THE VIRUS

Life's Enemy

BY

KENNETH M. SMITH, F.R.S.
DIRECTOR, PLANT VIRUS RESEARCH STATION, CAMBRIDGE

It is abundantly evident that a proper understanding of virus diseases and viruses is essential for the future well-being of mankind

SIR PATRICK LAIDLAW

CAMBRIDGE
at the University Press
1942

EDWARD JENNER

1749–1823

From the picture by James Northcote, R.A., in the National Portrait Gallery, photographed by Messrs Emery & Walker, Ltd., London.

Dedicated to

CARL TENBROECK, M.D.

and his colleagues of the
Rockefeller Institute, Princeton, U.S.A.

in appreciation of much kindness
and hospitality

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CHAPTER I

THE DISCOVERY OF VIRUSES

MEN have always been afflicted with epidemic diseases; but, before the era of scientific investigation, the causes to which they were attributed were fantastic in the extreme.

A favourite superstition was that epidemics were due to the destruction of heavenly bodies, the ashes of which fell upon the earth. Other beliefs were vested in the vengeance of malignant deities, the influence of the moon and so forth. And such superstitions are still held by peasants in isolated country districts even up to the present time. For instance, in the central province of France one summer day just previous to the outbreak of the Great War (1914), a farmer was working in his fields when he was taken suddenly ill. The peasants nearby hurried to his assistance, and later, one of them commenced to dig in the ground at the spot where the farmer was found. "For", he said, "if we dig deep enough, we shall find the salamander."

In this introductory chapter then we shall begin, not quite at the age of superstition, but at the time of the first realizations that very small living things existed. From there we shall trace, step by step, the various discoveries and experiments which have made possible the conception of such a disease agent as the *virus* which is discussed in the rest of the book.

Leeuwenhoek's "very wee animals".

At the beginning of the seventeenth century there lived in Italy a Jesuit, Athanasius Kircher, who was one of the first to use a simple microscope and by this means to get a peep through the door leading to a new world, the world of the infinitely small. Kircher found countless numbers of minute "worms" in various putrefying substances which he examined with his simple lenses.

Later, in 1656, when the plague was raging in Italy, Kircher examined the blood and secretions of plague patients and again reports the discovery of innumerable "worms". In this case the "worms" were probably merely blood corpuscles and leucocytes, but the first step had been made leading away from the old superstitious reasons for infectious disease and towards a rational theory of "*contagium animatum*".

At about this time Robert Boyle in Ireland was writing with prophetic insight regarding the causation of infectious diseases. In his essay on the Pathological Part of Physic he wrote: "and let me add that he that thoroughly understands the nature of ferments and fermentation shall probably be much better able than he that ignores them to give a fair account of diverse phenomena of several diseases (as well as fever and others) which will perhaps be never properly understood without an insight into the doctrine of fermentation." In commenting on this Nuttall says: "In those words written some 250 years ago, we trace the beginnings of what became the 'germ theory' of disease."

The man, however, who did more than any other to pave the way for a scientific study of minute organisms was Antony van Leeuwenhoek, a Dutch linen draper, born at Delft in Holland in the year 1632.

Van Leeuwenhoek, who is regarded as the father of the modern microscope, made during his lifetime more than 250 different kinds of microscopes. (Some of these were bequeathed to the Royal Society, but the collection was unfortunately lost.) Van Leeuwenhoek taught himself the art of grinding lenses which magnified from 40 to 160 diameters and even to 300 diameters and as his interest in this work increased, he gradually subordinated his drapery business and gave himself up to the study of micro-organisms.

Many of his observations are recorded in letters addressed to the Royal Society, and one of these, dated 9 October 1676, contains what is probably the first recorded description of bacteria. This famous letter, which is quoted from Dobell's charming book, *Antony van Leeuwenhoek and his little animals*, published in 1932, describes how an infusion was made of pepper in water and the mixture allowed to stand in an open vessel for about 3 weeks. After this period van Leeuwenhoek examined the infusion with his microscope and states: "I saw therein, with great wonder, incredibly many very little animalcules, of divers sorts." These were probably mostly Protozoa, but there was another kind of "little animal" which is described as follows: "The fourth sort of little animals, which drifted among the three sorts aforesaid, were incredibly small; nay, so small, in my

sight, that I judged that even if 100 of these very wee animals lay stretched out one against another, they could not reach to the length of a grain of coarse sand; and if this be true, then ten hundred thousand of these living creatures could scarce equal the bulk of a coarse sand-grain" (=approximately $\frac{1}{30}$ inch in diameter). There is little doubt that here van Leeuwenhoek is describing bacteria and that he and Kircher were the first men to see those living cells which are too small to be seen with the naked eye.*

After this invention of the microscope and the discovery of these minute living things, the development of the science of bateriology was surprisingly slow. It was nearly 200 years later that Pollender in 1849 discovered the anthrax bacillus, which was the first bacterium to be definitely associated as a causative agent of an infectious disease. Some years after this, additional evidence in support of the "germ theory of disease" was afforded by the work of Robert Koch, who, in 1878, described six different types of infective diseases which could be produced by injecting putrid fluids into animals. Koch showed that these diseases varied in symptoms and pathology and that the bacteria were different in form and distribution. From these early discoveries has arisen the comparatively new science of bacteriology which we have called—*the world of the infinitely small*. We come now to consider the subject of this book, *the viruses*, and these we may appropriately call *the world of the infinitely smaller*.

* For a table illustrating the comparative size of Protozoa, bacteria, viruses and protein molecules, see p. 41.

Smallpox, a virus disease.

The term virus is an old one derived originally from the Latin meaning "poison". The *Oxford Dictionary* gives a choice of three examples, as follows: "Cleopatra pouring the virus of an asp into a wound in her arm"; "Venice is a stinkpot charged with the very virus of hell"; and finally, "The pustules contain a perfect smallpox virus." The first of these uses the word literally as meaning a poison, the second may perhaps refer to what is known in America as moral turpitude, while the third describes the particular type of agent with which we are concerned. And before we come to the recognition of the viruses themselves, we ought to refresh our memories about the disease of smallpox, a typical virus disease.

In a churchyard at Worth Matravers there is a tombstone with the following epitaph: "Sacred to the memory of Benjamin Jesty of Downshay, who departed this life April 10th, 1810, aged 79 years. He was born at Yetminster in this county and was an upright honest man particularly modest for having been the first person known that introduced the Cow Pox by inoculation and who from his great strength of mind made the Experiment from the Cow on his wife and two sons." Although it may be true that this modest pioneer was one of the first to practise vaccination, there is no doubt that it is to Jenner that mankind owes the immense benefits which have accrued from the discovery. The physician Edward Jenner was born in 1749 and died in 1823 at

Berkeley, Gloucestershire, where he spent the greater part of his life. Owing to a local belief that dairymaids escaped smallpox, he sought to test this belief by experiment. The results of his first experiment, made on 14 May 1796, are best told in a letter from Jenner himself to his friend Gardner, quoted from Baron's *Life of Jenner*:

DEAR GARDNER,

As I promised to let you know how I proceeded in my inquiry into the nature of that singular disease the Cow Pox, and being fully satisfied how much you feel interested in its success, you will be gratified in hearing that I have at length accomplished what I have been so long waiting for, the passing of the Vaccine Virus from one human being to another by the ordinary mode of inoculation. A boy of the name of James Phipps was inoculated in the arm from a pustule on the hand of a young woman who was infected by her master's cows. Having never seen the disease but in its casual way before; that is when communicated from the cow to the hand of the milker, I was astonished at the close resemblance of the pustules, in some of their stages, to the variolous (smallpox) pustules. But now listen to the most delightful part of my story. The boy has since been inoculated for the smallpox which, as I ventured to predict, produced no effect. I shall now pursue my experiments with redoubled ardour.

Believe me yours very sincerely

EDWARD JENNER.

Berkeley, July 19, 1796.

Baron states that prior to the advent of vaccination it has been calculated that one in fourteen born in London died of smallpox, and of persons of all ages taken ill with smallpox, one in five or six died. In the Russian Empire

the disease is reported to have been so malignant as to have cut off 2,000,000 inhabitants in a single year, while Lettsom calculates that 210,000 fell victims to it annually in Europe. In the space of 7 years smallpox is said to have been imported more than 100 times into the English Channel, and in the year 1800, twenty times by the Channel Fleet alone.

The ladder of experiments which rejected the theory of spontaneous generation.

It is not clear from his writings whether Jenner had any idea in his mind as to what was the actual agent that produced smallpox in man or cowpox in animals. It must be remembered, however, that Jenner's work was performed years before anyone had proved there was any connection between infectious diseases and bacteria; so that it is not surprising if he was unable to visualize the existence of a virus, so much more elusive an agent. We can, however, learn something of Jenner's views on the causative agent of another virus disease, herpes. In one of his letters he says: "One word more on herpes. Seeing how frequently the vaccine disease becomes entangled with it, my thoughts have been pretty much bent upon it and *I now see that the herpetic fluid is one of those morbid poisons which the human body is capable of generating, and when generated, that it may be perpetuated by contact.* Children who feed on trash at this season of the year are apt to get distended bellies, and on them it often appears about the lips. This is the most familiar example I know. A single vesicle is capable of

deranging the action of the vaccine pustule. Subdue it and all goes on correctly."

This letter contains two points of interest to which we shall have occasion to refer later in this book, one is the effect of one virus upon another operating in the same host, and the other is the possibility of a virus arising spontaneously or, as Jenner puts it, "a morbid poison which the body is capable of generating". The superstition of spontaneous generation, that is, the production of living things from non-living matter, dates from remote antiquity. The bible, for example, instances the birth of bees from the skin of a dead lion. This superstition lasted for many centuries and was only gradually dispelled by the careful experiments of a number of scientific workers. As the apparent production of one type of animal from non-living matter was shown to be erroneous, the belief attached itself to another form still lower in the scale of life. Thus when it was demonstrated that the recipe of mixing dirty old clothes with corn in a glass vessel did not really give rise to adult fully-formed mice, people still believed that putrid meat generated maggots. When Redi shattered this belief, there was still the case of the insect in the gall which was thought, like the gall itself, to be a product of the plants; then for many years the idea persisted that bacteria and other micro-organisms were generated by decomposing matter. If we now ascend, step by step, this ladder of experiments, each one excluding a smaller and smaller organism, we shall arrive at the discovery of the first virus.

Since the most distant times it has been noticed that organic matter, if left exposed to the air, decomposes and may become covered with moulds or filled with maggots. It was Pasteur who discovered the explanation of fermentation; but it is to Redi, 200 years earlier, that the credit is due for giving the true explanation of the appearance of maggots in decomposing matter. Francesco Redi was born at Arezzo in Tuscany in 1626 and died in 1697. It was in 1668 that he carried out his famous experiments which not only disproved the theory of the spontaneous generation of maggots but also made known at the same time the method of development of insects. Under present-day conditions Redi's experiments seem simple enough, but in the middle of the seventeenth century it must have required much courage and authority to demonstrate a truth which went against the scriptures. Redi showed that if pieces of meat or dead animals are exposed to the air during hot weather, maggots soon make their appearance in the flesh. If, however, the same material is placed, when still fresh, in a jar covered with a fine gauze no maggots appear. It is thus clear that the maggots are not engendered by the corruption of the meat but by something which arrives from outside, in other words flies. Redi also observed the flies deposit their eggs on the gauze, and further established the connection between the two by confining a piece of maggoty meat in a jar covered with gauze until the appearance of flies derived from the maggots.

It is rather remarkable that after his own demonstra-

tion that maggots in meat are not spontaneously generated, Redi himself should still have believed that the fly which causes galls on certain plants was the product of the plant and did not pass through a series of changes similar to those of the meat flies.

The true explanation of the formation of galls in plants was given by Vallisnieri about the year 1700. In spite, however, of the experiments of Redi and others it was still the general opinion that spontaneous generation was the rule regarding the bacteria and other micro-organisms which had been revealed by the simple microscopes of Kircher and Leeuwenhoek. We come thus to the last rung but one of our ladder, the exclusion of micro-organisms from infusions of organic matter, much more difficult experiments than those of Redi with the flies. These experiments, however, when completed finally disposed of the belief in spontaneous generation and the hypothesis that all living matter must arise from pre-existing living matter (*omne vivum ex vivo*) obtained its establishment in science. In his *History of Bacteriology*, Professor Bulloch describes in great detail the many and varied experiments carried out by different workers to solve the problem of the origin of micro-organisms in organic infusions and much of this information is obtained from his fascinating account. The Dutch microscopist, van Leeuwenhoek, did not believe in the spontaneous generation of his "little animals" but thought that they came in the air. In 1710 Louis Joblot demonstrated that the animalcules did not arise from the matter of the infusion itself; he boiled hay infusion for

15 minutes and then poured it in equal quantities into two vessels, one of which he sealed while hot and the other left open to the air. After a time organisms appeared in the latter vessel but not in the sealed flask. Joblot then removed the seal and exposed the contents to the air and in a short time this infusion also contained micro-organisms.

In spite of this experiment, however, the doctrine of spontaneous generation persisted and the next champion to enter the field was the Italian naturalist Spallanzani, who published two accounts of his researches in 1765 and 1776. Spallanzani carried out a great many experiments in which he used different kinds of flasks and sealed them in different ways. His conclusion was that it was not enough to seal the flask hermetically, the air in the flask must contain no animalcules or their "germs". An important variation in the experimental method was introduced in the middle of the nineteenth century by Schröder and von Dusch, who freed the air in their flasks from organisms by forcing it through a tube of cotton-wool instead of heating it. This was the first time that the technique of "filtering" was used for purification, and it is a process which has a great significance for the study of the disease agents which we shall later discuss and which are known as "filterable viruses".

About 1860 Pasteur took a hand in this work and demonstrated not only that the air really does contain micro-organisms but also that a sterile infusion becomes contaminated if dust, the deposit from air, is introduced

into it. Pasteur also showed that boiled infusions may be kept sterile in open flasks, provided the neck of the flask is drawn out and bent down in such a way that germs from the air cannot ascend. Some of Pasteur's flasks which he rendered sterile in 1860 have been preserved and are still sterile after nearly 80 years.

However, in spite of all the precautions taken by these many investigators, a percentage of the flasks continued to develop growths of bacteria, and this fact was seized upon by the supporters of the theory of spontaneous generation. The final blow to this doctrine was given by the English physicist, John Tyndall, who showed that bacteria have phases, one being easily destroyed by boiling (this is known as the thermo-labile stage) while the other (the thermo-resistant or spore stage) would resist heat to an almost incredible degree. Tyndall also devised the method of fractional sterilization by discontinuous heating, which is known to-day as Tyndallization. Tyndall showed that discontinuous boiling for 1 minute on five successive occasions would sterilize an infusion which resisted a single continuous boiling for 1 hour. This technique is based on the observation that active bacteria are easily killed by boiling, and a certain period of time is necessary to allow for the formation of the heat-resistant stage. We may appropriately take leave of this problem by quoting a sentence of Professor Bulloch, "the result of all the work above described and carried out by so many workers sufficed to lead to the firm belief that spontaneous generation is in reality what Pasteur called it—a chimaera".

Thus the ghost of spontaneous generation, or heterogenesis as it is called, appeared to be laid for ever; the reader will be surprised to learn that the idea is once again being discussed with regard to the viruses and in Chapter III a series of experiments is described which bears a striking resemblance to those we have just detailed. The idea, as we shall see, however, has become considerably more subtle.

The discovery of viruses.

We come now to the last rung of our experimental ladder, and at the same time we leave the micro-organisms and pass over into the still uncharted country of the filterable viruses. We have seen how Redi excluded flies with a fine gauze and how Schröder and von Dusch kept out bacteria with cotton-wool plugs. From these early attempts have arisen other methods of exclusion such as the experiments of Tiegel, who in 1871 filtered anthrax fluids through a porous cell of unburnt clay, and those of Pasteur and Joubert in 1877 who employed plaster of Paris to separate anthrax bacilli from their containing fluids. Chamberland, working in Pasteur's laboratory, devised a filtering apparatus of candle shape of unglazed porcelain, and this apparatus, known as the Pasteur-Chamberland candle, is still in use at the present time.

In 1891 Nordtmeyer introduced a new filter medium made of the compressed infusorial earth known as Kieselguhr. The filtering capabilities of this substance were noticed because of the fact that the ground water

in the Kieselguhr mine in Unterluss (Hannover) was of a clear blue colour. The filter of Kieselguhr was called Berkefeld, from the name of the owner of the mine. Both the Pasteur-Chamberland and the Berkefeld filters are still widely used. Such filters are capable of holding back the smallest micro-organisms which it has been possible to see with the highest power of the microscope; the fluids which have passed through these filters are sterile; that is, there are no bacteria or other micro-organism present. The reader may think that here we have surely reached the lowest limit of size of a living organism. That may well be so. But, nevertheless, we have not nearly reached the limits of size of those agents of infectious disease known as viruses.

In 1892 Ivanovski, a Russian botanist, was investigating a disease of the tobacco plant known as tobacco mosaic (the disease is so called because the leaves are mottled in a light and dark green pattern). He extracted some of the sap of such a diseased plant and passed it through a fine filter candle; he then discovered that the filtrate, although sparkling clear and free of all bacteria, yet had the power of producing the mosaic disease when rubbed on to the leaves of healthy tobacco plants. This was the first scientific demonstration of the existence of a virus disease.

Little attention was paid to this remarkable discovery at the time; Ivanovski himself did not realize its importance, and still thought the disease was caused by bacteria, despite the evidence of his own experiment. The phenomenon was rediscovered about 7 years later

by Beijerinck who introduced his theory of a living infectious fluid (*contagium vivum fluidum*), a conception which is still not disproved. In 1898 two workers, Loeffler and Frosch, working with the well-known foot-and-mouth disease, demonstrated that the agent causing this disorder was also capable of passing a bacteria-proof filter. After these first discoveries progress, as in the early days of bacteriology, was slow, and it is only within the last two decades that the importance of the viruses has been recognized and these agents have received the attention they deserve.

Virus diseases.

Although the scientific demonstration of the existence of viruses is of such recent date, the diseases they cause have been known for centuries, and references to them can be found far back in history. We have seen already that Jenner, in his work on vaccination against smallpox, was dealing with a true virus disease long before the connection between the visible bacteria and infectious disease was known.

Zinsser has gathered evidence concerning early epidemics of infectious diseases and suggests that it is probable that smallpox existed in China as early as 1700 B.C. By the year A.D. 1000 it was present in practically all European nations, and after the discovery of America the disease was imported by the discoverers and spread rapidly among the natives. Zinsser relates how in the conquest of Mexico, a negro from the ship of Narvaez carried smallpox ashore and over 3,000,000

Indians are said to have died of it. By the middle of the sixteenth century the entire world had become infected with the virus.

The virus disease called yellow fever has been known since early in the seventeenth century, and is thought to have been confined to the Western Hemisphere until connection between the two hemispheres was established by Columbus. During the French Revolution, when the quarantine of European ports was suspended, the virus spread into Europe and the remaining parts of North and South America. The establishment of the Haitian Republic is largely due to yellow fever, since of 25,000 French troops sent to invade the island, 22,000 died of the disease.

The earliest known reference to a plant virus was published in 1576 and concerns an infectious variegation in tulips now called "tulip-break". Illustrations of tulips which show typical symptoms of this disease are to be found among the works of Dutch artists about the time of Rembrandt.

To summarize the practical importance of viruses: in the preceding pages we have already made the acquaintance of the virus diseases smallpox and yellow fever, and to these may be added measles, mumps, chicken-pox, epidemic influenza, infantile paralysis and trench fever. Animals and birds suffer from many virus diseases such as foot-and-mouth disease, dog distemper, swine fever, louping-ill, psittacosis or parrot fever, fowl plague and the allied pox diseases such as fowl-pox, pigeon-pox and canary-pox. Fish and insects

are also attacked by viruses, and even bacteria themselves are destroyed by an agent having many of the properties of a virus and known as a bacteriophage. Plants of all kinds suffer from a great variety of virus diseases and no fewer than 135 separate plant viruses have been described.

The amount of suffering and loss of life caused to mankind by virus diseases is incalculable, and to this must be added the financial losses in animals and crops caused by the same type of disease agent. In fact, as Laidlaw remarks, the sum total of the disharmony that viruses produce rivals that caused by the visible bacteria. In the great pandemic of 1918–19, influenza destroyed more lives than did 4½ years of the Great War. In London alone it killed 18,000 persons and in India nearly 6,000,000. The recent epizootic of foot-and-mouth disease has cost the Continental countries several millions of pounds, and the losses in this country, though less than that owing to the rigorous policy of destroying infected animals, were bad enough. The annual loss to the potato industry by virus diseases is computed to be about £2,000,000. Similar losses due to virus infection are experienced in other crops, such as sugar beet, sugar cane and cotton, while virus diseases are spreading to an extent not realized in all kinds of ornamental and horticultural plants. This last fact is particularly serious, since so many ornamental plants are, like the potato, vegetatively propagated, and this of course ensures also the propagation, and incidentally the distribution, of the virus.

CHAPTER II

HOW THE VIRUS ITSELF IS STUDIED

The size of viruses.

In some of the virus diseases of plants the symptoms produced may be striking and unusual, resulting in certain cases in the development of numerous concentric rings on the leaves. On observing some of these rings a non-scientific friend exclaimed to the writer, "I thought you said viruses were invisible to the eye", and on receiving the assurance that they were, replied, "But I can see them", pointing at the same time to the rings on the leaf of the affected plant. This story illustrates the difficulty experienced by the layman in grasping clearly the difference between the *virus* and the *virus disease*, the two not being at all the same thing. It is possible, in the plant virus world at all events, to have one virus which may produce half a dozen different diseases according to the kind of plant it happens to infect. In the animal viruses also, the same virus may produce a different disease depending on the particular organ affected. In this chapter, then, we are not at all concerned with disease but only with the virus, how it can be isolated and how its properties and behaviour can be studied.

When Ivanovski in 1892 passed the sap from a mosaic-infected tobacco plant through a filter candle and found the virus still present in the filtered sap, he demonstrated one of the outstanding characteristics of

viruses, i.e. the intrinsic property, arising from their minute size, of passing through the pores of bacteria-proof filters. From that time on, these disease agents have been known as "filterable viruses". As we shall see, however, the term filterable is purely a comparative one and begins to lose its meaning with advances in the technique of filtering. At the present time, although most viruses are too small to be seen, even with the best compound microscope, it is nevertheless possible to measure the size of the actual virus particle; one method of doing this is by the use of a special type of filter. This method, briefly, consists of passing the fluid which contains the virus through pores of a known diameter; the size of the virus particle can then be calculated from the diameter of the pore which just fails to let the virus pass. This procedure sounds simple enough, but in reality it is a difficult and exacting technique.

The kind of filter candle made of fine porous clay such as that used by Ivanovski is obviously unsuitable for this sort of work, since the porosity is made up of holes of many different sizes and the fine channels are not straight tubes but ramify in all directions through the thick walls. In the special filters, one has to aim at straight tubes of uniform sizes. The material used in preparing them is collodion, but it must be treated in the particular manner devised by Dr W. J. Elford. Collodion films have been used for filtration purposes for a number of years; they are of two kinds, one prepared with acetic acid-collodion and the other with ether-collodion. Elford has shown that collodion films

exhibit two types of microscopical structure, one very coarse and containing pores of many different sizes and one fine and uniform but too impermeable for use in filtering. These two types of structure co-exist in the acetic acid-collodion films, while the ether-collodion films exhibit only the fine impermeable structure. It is clear therefore that these collodion membranes are too irregular in structure to permit of any accurate measurements. Now Elford found that amyl alcohol and acetone were mutually antagonistic in their solvent action towards nitro-cellulose, and the presence of both in the same solvent medium produces coagulation of the nitro-cellulose in a uniform manner: thus the pores in a given membrane are all of the same size. This phenomenon has been used to produce the ultra-filter membranes which are known as Gradocol membranes, because of the graded coagulation of the collodion.

When the membranes have been prepared, the next step is to standardize them or, in other words, to measure the size of their pores. To do this it is necessary to have three sets of data, i.e. the water content of the membrane, the thickness and what is called the rate of flow of water. This means the time taken for a given quantity of water to flow through a given area of the membrane. When the necessary data have been obtained it is then possible, by means of a simple formula, to calculate the average pore diameter of the membrane.

To measure the particle size of a virus, the clarified fluid containing the virus is filtered under pressure through a series of membranes, in descending order of

pore size, until the filtrate no longer contains the virus, a fact which is demonstrated by inoculation to a suitable susceptible organism. The particle size of the virus in question is then taken to be a certain fraction of the pore size of the membrane.

By means of this technique the particle sizes of a number of animal viruses have been measured and a representative series is given in Fig. 7. It will be seen that the smallest virus of all is that of foot-and-mouth disease which measures only 10 millimicrons in diameter, while the largest is psittacosis virus with a diameter of 275 millimicrons. A micron is a thousandth of a millimetre and a millimicron is a millionth of a millimetre. This figure also illustrates the sizes of the smallest bacteria (which are unquestionably *living* entities, by my definition) and the largest protein molecules. It is important to observe at once how the sizes of viruses overlap into both the protein and bacteria regions.

In carrying out filtration experiments of the above nature, all sorts of other complicating factors come in, such as the questions of clearing the virus fluid of other materials, which block up the pores of the membrane, the *p*H or degree of acidity of the fluid, the electric charge on the particles and even their shape. Now in the ultra-filtration of plant viruses we have all these difficulties in an aggravated form, and chief among them is the fact that many of the plant viruses are not spherical but rod-shaped. This question of the shape of the virus particle is of great importance from the filtration point of view, not only because the calculations of

the particle size are based on the assumption that the particle is a sphere, but also because a rod is not able to pass through a pore so easily. By stretching the imagination slightly one can perhaps liken these rods to logs going down a narrow stream, and it is easy to see that jamming and blocking are more likely to occur with a crowd of logs than with an equal number of footballs. The reasons for supposing some of these plant viruses to be rod-shaped will be dealt with later in this chapter.

Ultra-filtration experiments with plant viruses have shown that the particle size of two, which we have reason to believe are spherical and nearly spherical, lies between 17 and 25 millimicrons. Such a size indicates that these are among the smallest viruses.

One method then of measuring the size of virus particles is by ultra-filtration, of which we have sketched the barest outline. There are several other methods of estimating the size of viruses some of which are the result of developments in physical technique.

Although it is not possible to see the majority of viruses with the microscope, some of them can be photographed and therefore measured under certain conditions. By using single wave-lengths in the ultra-violet spectrum and a special type of microscope fitted with quartz lenses, Barnard has photographed several animal viruses and measured their particle diameters. The values obtained with the ultra-violet light microscope are in close agreement with the ultra-filtration measurements; for example, both methods give values of 125–150 millimicrons for the viruses of vaccinia,

Fig. 1. The virus of fowl plague. ×2250. The size of the particles varies from 90 to 100 millimicrons.

Fig. 2. Vaccinia virus. ×2250. The size of the particles is about 150 millimicrons.

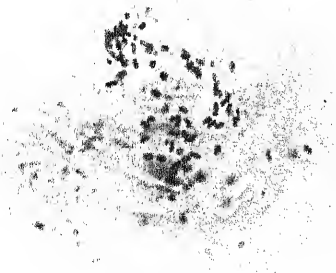


Fig. 3.

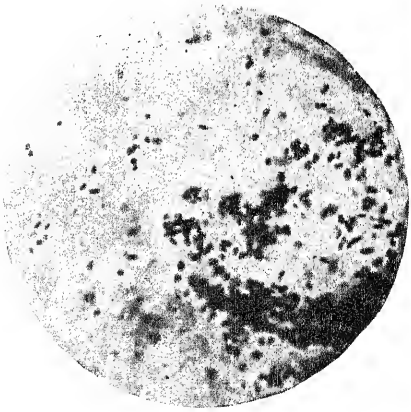


Fig. 4.

Figs. 3, 4. The virus of foot-and-mouth disease. ×3200. The smaller particles measure only about 40 millimicrons.

(1 millimicron equals 1 millionth of a millimetre)

(*Ultra-violet light photos by J. E. Barnard*)

canary-pox and infectious ectromelia of mice. The microscopy of viruses has recently been greatly advanced by the invention of the electron microscope. In this apparatus a magnetic field acts on electronic rays in a vacuum, and "magnetic lenses" are used much as optical lenses in the ordinary microscope. By this means magnifications up to about 20,000 can be obtained.

Another method of measuring the size of particles, viruses included, is by means of the ultra-centrifuge, an apparatus which spins the particle-containing fluid at very high speeds. The size and molecular weight of the particles can be calculated from the rate at which they sediment or pack down on the bottom of the tube. Again, it is possible to measure viruses by means of X-ray photographs, which have recently given dramatic results in many virus problems. Although there is a general agreement in the results obtained by the various means of measuring the particle size of viruses, yet there are still discrepancies; it is probable that these are due to the fact that some of the viruses are not spherical. The calculations used in the ultra-filtration and centrifugation methods are based on the supposition that the particle is a sphere, and so errors are liable to arise in the case of rod-shaped viruses.

The destruction and extinction of viruses.

We know that bacteria are rapidly destroyed by certain physical and chemical agents such as heat and antiseptics. These agents act in a rather similar way upon viruses. For example, all viruses can be destroyed

by heat: but there is a very wide variation in the temperature at which the different viruses are destroyed. In the plant viruses this temperature, or thermal-inactivation point as it is called, ranges from as low as 40° C. to as high as 90° C. for 10-minute exposures, and there is a wide range also in the temperature reactions of the animal viruses.

Some viruses are easily destroyed by alcohol, while others are much more resistant; there is, for example, one plant virus which can be kept in absolute alcohol on the laboratory bench for 6 months and still retain its infective power.

Drying, or desiccation, destroys some viruses but not all; the virus of tobacco mosaic will remain infective for periods of years in dried leaf tissue, and this is one reason why the virus can be found still infective in cigars and cigarettes. It will be recalled that the virus of foot-and-mouth disease can also withstand drying, which makes possible the suggestion that the virus is brought into this country on the feet of birds. It could also survive for a time in dried milk powders were it not for the fact that probably all milk is pasteurized before it is dried and that alone would destroy the virus. The virus of tobacco necrosis, which is located in the roots of tobacco plants, is very resistant to drying; this enables it to be carried in the air in dried particles of plant tissue and so to reach new host plants. Yellow-fever virus also when dried and frozen can be kept in an active state for many months.

There is great variation in the time for which viruses

will retain their infectivity outside the host. We have mentioned above that the virus of tobacco mosaic will remain infective in the dried state for several years. This longevity, or resistance to ageing, is, however, bound up with several other factors connected with the medium in which the virus is suspended. The rapid oxidation which takes place in sap when extracted from the plant causes the destruction or inactivation of many plant viruses. Some of these, however, remain infective for long periods if they are separated from the various plant products and are isolated in a pure state. We shall see shortly how this can be accomplished.

Irradiation with ultra-violet light destroys bacteria and also both plant and animal viruses; there is a difference, however, in their respective reactions because the viruses are able to withstand a greater exposure to the rays than bacteria or even their vegetative spores. The resistance ratio of virus to bacteria is considered to be about 200 : 1.

These facts about the destruction of viruses become important when one is trying to extract them. When a virus is extracted from its host, whether animal or plant, it is accompanied by a quantity of other substances which may profoundly affect the behaviour of the virus. Therefore in order to study the true nature of viruses it becomes necessary to free the virus from the extraneous proteins, etc., which accompany it in plant or animal extracts. How can this be done? The answer has been given by the biochemist who has applied his special methods to the problem. The first steps in this direction

were made some years ago by two American workers, Vinson and Petrie, who showed that tobacco-mosaic virus could be thrown down, or precipitated, out of suspension by the use of well-known protein precipitants, such as ammonium sulphate. They also showed that the virus could be precipitated by means of safranin and that a complex was formed of virus and safranin in which the virus was inactive. If the safranin was removed, however, by dissolving it in amyl alcohol, then the virus was regained in an infective state. This work was important because it showed the virus in a new light—*as behaving like a chemical substance rather than an organism.*

In 1935 a further and important step forward was made by an American, W. M. Stanley, working at the Rockefeller Institute in Princeton. Stanley applied the chemical methods of "salting out", by which enzymes have been prepared in a pure crystalline state, to suspensions of tobacco-mosaic virus. By these means he obtained a protein of high molecular weight which had all the properties of the virus itself.

When the sap of a mosaic-diseased tobacco plant is extracted by grinding in a mortar it consists of a thick brownish green fluid. This fluid can be cleared of all visible particles by filtering it through a bed of diatomaceous earth or similar substance; it then appears as a clear brown sparkling liquid. Now if the *p*H of this fluid is adjusted to 3·3, or in other words, if the fluid is brought to a particular degree of acidity by the addition of a few drops of weak hydrochloric acid, a marked change takes place. A cloudiness first develops and this

is followed by the appearance of a characteristic precipitate with a curious shimmer or sheen. This precipitate is the virus and constitutes the first step in the purification process.

After further treatment, involving the use of ammonium sulphate and certain other technical processes which need not be detailed, the apparently pure virus protein is obtained. Although this protein was thought at first to be truly crystalline, it was discovered later not to be so but to form paracrystals or "microtactoids". This means that the protein has some of the orderly arrangements of crystals but in a two-dimensional, instead of a three-dimensional form. The actual crystallinity is not, however, of very great importance; the significant fact is the isolation of a pure chemical substance which appears to be the virus itself.

When the isolation of this protein was first announced, it was suspected that the paracrystals were not the actual virus but merely carried it contained within them. There is now, however, a great deal of evidence against this hypothesis. The abnormal protein can be isolated from any plant which is susceptible to infection with the virus, and such plants may, botanically speaking, be far removed from the tobacco plant. It cannot therefore be something peculiar to the tobacco. Any factor, such as temperature or acidity, which degrades or destroys the protein reduces or destroys the infectivity of the virus. Again, the protein gives a sharp sedimentation boundary on the ultra-centrifuge: this would not be the case if it was a mixture of substances. In other words, if there is

a second substance mixed with the virus then it is necessary to assume that both have the same chemical and physical properties and, since there is a sharp sedimentation boundary, the same high molecular weight of about 17,000,000. The virus protein, also, shows an extreme degree of infectiousness and will regularly infect at a concentration of 10^{-10}.

Thus the virus must be regarded as a protein. This is one of the most remarkable biological discoveries of recent years: for in many properties, such as reproduction, the virus behaves unquestionably as a living organism. The implications of this discovery are discussed in Chapter III.

The virus proteins.

Unusual proteins have been obtained from other plant viruses, and Bawden and Pirie have shown that the virus causing bushy stunt disease in tomatoes can be crystallized *in true three-dimensional form*. Similarly, recent work on purification of the tobacco-necrosis virus has produced large plate-like crystals which we have every reason to believe are the actual virus. Proteins of high molecular weight but non-crystalline have also been isolated from the tissues of animals infected with viruses such as papillomata of rabbits and encephalomyelitis of horses. Even the bacteriophage, which is suspected to be a virus attacking bacteria, has yielded a protein of molecular weight approaching 500,000 which, in quantities so small as 1×10^{-12} milligram, will destroy some bacteria.

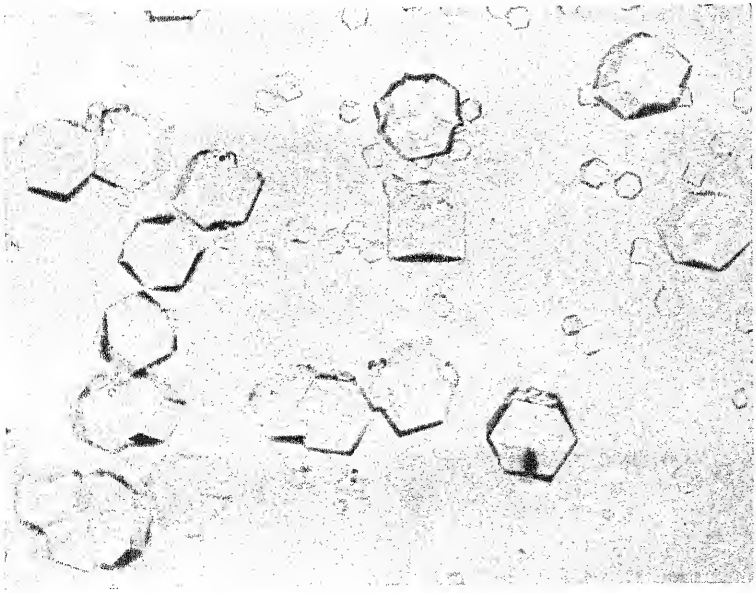


Fig. 5. The virus of tomato bushy stunt in its crystalline form.
(*After Bawden and Pirie*)

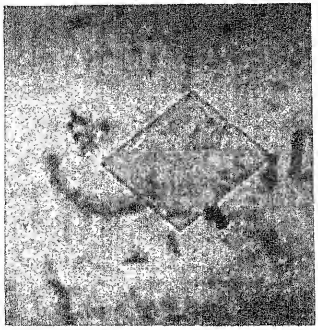


Fig. 6. The virus of tobacco necrosis in its crystalline form.

We have seen that virus in an apparently pure state can be obtained from the sap of a virus-diseased plant by chemical methods of purification. Another method of obtaining purified virus is by the use of the ultra-centrifuge by means of which the virus sap is spun at very high speeds, thus sedimenting the heavy virus proteins at the bottom of the tube. When a sample of tobacco-mosaic sap is centrifuged for 3 hours at 25,000 revolutions per minute the heavy virus protein is thrown down to form a fibrous crystalline mass at the bottom of the centrifuge tube.

The shape of the virus particles.

Mention has been made of the unusual white precipitate which is thrown down when the extracted sap of tobacco-mosaic plants is acidified. If a test-tube containing this precipitate is shaken with a circular motion of the hand, the precipitate will exhibit a curious movement in the reverse direction when the tube is once more at rest. This suggests that the precipitate consists of long rod-like particles joined end to end, which tend to untwist themselves.

The first suggestion that the particles of tobacco-mosaic virus were rod-shaped was made in 1935 by Takahashi and Rawlins, who observed that suspensions of this virus exhibited the phenomenon known as "anisotropy of flow". This simply means that when a fluid, containing a large quantity of submicroscopic elongated particles, is agitated, the elongated particles tend to come together and orient themselves with the long axes

parallel. The solution becomes birefringent. This birefringence persists when the concentration of the particles is high and the rods cannot move about freely; but in lower concentrations the birefringence only becomes apparent when the fluid is agitated and "streaming" or currents are set up. Bawden and Pirie have demonstrated this phenomenon by putting a goldfish in a weak suspension of purified virus; when the fish moved its tail it set up eddies in the fluid, thereby causing the particles to arrange themselves in parallel bundles so that birefringence was apparent in the region near the tail.

The interpretation of X-ray photographs (itself a highly technical process developed by Bernal for large molecular groupings) also indicates that the particles are elongated, having a width of about 150 Å., or 15 millimicrons, and a length of about 10 times the width.

Now although these methods give an accurate indication that certain virus molecules are elongate, it will be interesting to see how some confirmatory, though more circumstantial, evidence on the shape of virus particles can be obtained from other techniques. For example, the virus of tobacco necrosis is thought to be somewhat elongated in shape, though not to the same extent as the tobacco-mosaic virus. In filtering the tobacco-necrosis virus through the graded collodion membranes previously described, it has been observed that the virus passes steadily through membranes of average pore diameter of 1500 to 250 millimicrons. Between the sizes of 250 and 125 millimicrons, however, there is a sharp

drop in the virus concentration in the filtrates; and thereafter the virus passes the filters in diminishing quantity till it reaches a pore size of 40 millimicrons where it stops altogether. It appears, then, as if there exist two phases of filtration, the one being a steady passage through membranes of coarser grade down to 250 millimicrons average pore diameter and the other a much slower passage through membranes of 125 millimicrons average pore diameter down to 40 millimicrons. Now it is possible to explain this phenomenon on the following assumption: we can suppose that the virus is able to pass through all pores down to size 250 millimicrons at any angle. In other words, the particle can pass through either lengthways or broadside on. Below this pore size, however, the particle cannot pass through unless it enters the pore with its long axis perpendicular to the membrane face. On this assumption we should calculate the *length* of the particle from the upper limit of pore size and the *width* or diameter of the particle from the lower limiting pore size. This indicates that the particle is about 17–20 millimicrons in diameter with a length of about four times this value.

In a later chapter we shall touch upon the serology of plant viruses, that is, the antibody formation and other reactions when, for example, plant viruses are injected into rabbits. Here, however, we are only concerned with the possibility of obtaining some evidence of the shape of virus particles from the serological reactions. When the antiserum obtained from the hyperimmunized rabbit is mixed with the antigen, in this case

virus, a precipitate is formed. Now, it is from the precise form of this precipitate that some hint of the shape of the virus antigen can be obtained. If the antigen is rod-shaped or like the flagellae of bacteria, then the precipitates form rapidly, are flocculent and bulky. If, on the other hand, the antigen is spherical, the precipitate settles slowly and forms a compact mass at the bottom of the tube.

Measurements of concentrations of virus.

During the purification and other studies of plant viruses which we have briefly described it is necessary, at intervals, to make tests of the virus content of the various samples. It is obvious enough, of course, that the quickest way, and in some cases the only way, to find out if there is virus in a given sample is to inoculate to a susceptible plant. The reaction of the plant will indicate whether virus is present or not in the sample. This procedure, however, will only give a positive or negative answer; it will not give a *quantitative* result. In other words, there will be nothing to show, for example, whether a certain purification method has succeeded in concentrating a given virus, or if the purified product differs in any way from the crude virus sap, a very important matter in testing whether the crystalline portions are truly the viruses. The solution to this problem in regard to some plant viruses has been given by a rather peculiar type of reaction on the part of particular plants to inoculation with the virus. Suppose, for illustration, we take the case of the tobacco-mosaic virus

and a particular species of *Nicotiana*, *N. glutinosa*. When the solution containing the virus is rubbed lightly over the surface of the leaf with the finger, or a glass spatula, dipped in the virus, the plant reacts to the inoculation in 3 or 4 days. However, instead of producing the typical mosaic mottling which is so characteristic of this virus on the tobacco plant numerous small circular spots develop on the inoculated leaves. These spots are known as "local lesions", and as there is no further spread of the disease it is possible to carry out a number of successful transmissions on single plants. When the virus suspensions are highly diluted so that the number of lesions is small, it is thought that one lesion represents the point of entrance of one infectious virus unit. On this assumption, therefore, by counting the numbers of lesions in each case, one can arrive at a rough estimate of the respective virus concentrations. The method is somewhat analogous to Koch's method of "plating" bacteria. It must be realized, of course, that this "local lesion technique" is only applicable to those viruses which produce clear local lesions on inoculation. Whether the virus afterwards gives rise to a systemic disease in the plant is not important so long as the initial lesions are distinct enough to be counted. There are one or two precautions which must be taken in carrying out this technique; the leaves should be rubbed with approximately equal quantities of virus and by a uniform method. In addition, it is necessary to carry out the inoculations on half the leaf only, the other half leaf being inoculated with the check or control virus sus-

pension. This is done in order to avoid errors which would arise because all the leaves of one plant do not necessarily react equally to inoculation.

We have stated above that under certain circumstances a single lesion on an inoculated leaf may be taken as representing a single virus unit. The word unit is used intentionally, as it does not necessarily imply a single virus particle. In studying the behaviour of such minute bodies as viruses and proteins it is necessary to take into consideration the tendency on the part of those bodies to aggregate or in other words to stick together in masses. This aggregation, which is partly dependent upon the electric charge of the particles, is more likely to occur with rod-shaped bodies than with those which are spherical. Now so far as we know an aggregate of virus particles will only produce one lesion and this fact is significant in interpreting the local lesion technique.

If after certain chemical treatment the number of lesions produced on the plant is much less than before such treatment, it does not necessarily follow that so much virus has been destroyed. It may mean only that the virus particles are clumped together and so would produce fewer lesions. It is sometimes possible to break down these virus clumps by dilution or by changing the degree of acidity of the solution. In the case of tobacco-mosaic virus, however, it is very difficult to disperse the aggregates, probably because of the rod-like shape of the individual particles.

CHAPTER III

WHAT IS A VIRUS?

The virus as a living organism.

In the preceding chapter we saw that a virus, extracted from its host, appears to be nothing more nor less than a protein of high molecular weight. On the other hand, we know that introducing one unit of such a virus *into* a susceptible host will cause the reproduction of millions of units of the virus. That is, the virus grows (though only in the living cell of its specific host).

These facts put us in a dilemma. Many people have asked the question, Are viruses living or non-living? This starts a hare which can never be caught, because there is no precise definition of a living thing or exact criterion of life. We cannot do better here than quote a remark made over 2000 years ago by Aristotle: "Nature makes so gradual a transition from the inanimate to the animate kingdom that the boundary lines which separate them are indistinct and doubtful." We may perhaps be allowed to put the question in a slightly different form and speculate whether the viruses form a series of which the more complex fall on one side, and the less complex on the other side, of the shadowy boundary which separates the living from the non-living.

The theories which have been put forward to explain the viruses fall into two categories: first, that which supposes a virus to be a micro-organism comparable

perhaps to a bacterium of extremely small size, and secondly, that which defines the virus as a chemical, possessing unusual properties, which may be the product of the disordered cell itself. In the former case a virus could be regarded as a degradation of the higher forms of organization and in the latter as a link between the higher and lower forms.

We propose to discuss first the suggestion that viruses may be very small micro-organisms. Since no virus has yet been cultivated outside a living cell, it follows that viruses, if they are organisms, must be extremely specialized parasites adapted only for an intracellular existence. They can only multiply in certain hosts and sometimes show preference for particular types of cells. The theory that viruses are a form of highly specialized parasitic organism which has developed parasitism to its utmost limit has been put forward independently by Gortner and Laidlaw.

The two essential parts of a living cell are the nucleus and its surrounding cytoplasm, and the functions of the nucleus may be considered as the ability to regenerate cytoplasm and thus to provide the appropriate environment for the nucleus's existence and also the ability to reproduce its own kind through the process of cell division. Gortner suggests that some of the more specialized parasites which are known to be organisms may have lost some of their synthetic functions and have to depend upon a particular host, manufacturing some special chemical necessary for the environment of the parasites' cell nuclei.

Now if we push this specialization a little further we can visualize a living parasite in which the cell nuclei have lost nearly all the synthetic functions necessary for the production of cytoplasm and have retained only those nuclear functions necessary for building nuclear material (chromatin) and for cell division (reproduction). In other words, we visualize what is virtually a naked nucleus which has adopted the host's protoplasm as its own cytoplasm and so would be wholly dependent upon the host for its nutrition, retaining only the power to reproduce more "naked nuclei". Such a parasite might also lose the phenomenon of respiration which we regard as essential to all living organisms but which is not shown by viruses.

Parasitism makes the parasite lazy, and the tendency develops more and more for the parasite to live a "borrowed life". Certain pathogenic bacteria, for example, lose their power to synthesize substances which are essential for growth and have to rely on the host to supply the missing substances. Since these bacteria live continuously in an environment where many of the factors for life and growth are preformed, they gradually lose their power of synthesis. Laidlaw's theory supposes that the larger viruses are organisms which have lost the power to synthesize some factor essential for growth or multiplication, perhaps a ferment or co-ferments. As we pass down the scale, the viruses diminish in size, and so we would postulate the loss of more and more factors essential for growth. The smallest viruses would therefore have lost all ferments and all auto-synthetic potenti-

alities, and could only multiply within the appropriate cell where they would find the necessary substances and ferment systems essential for their growth.

Laidlaw makes the interesting suggestion that the cellular damage caused by viruses may be in large measure due to the deviation of essential substances and ferments from their normal function in the host's cells. On this assumption, we would expect the smallest viruses to do most damage, since they, having lost more of their essential ferments than the larger viruses, would require more from the host cell. So far as the plant viruses are concerned, however, there is not much evidence either way on this point.

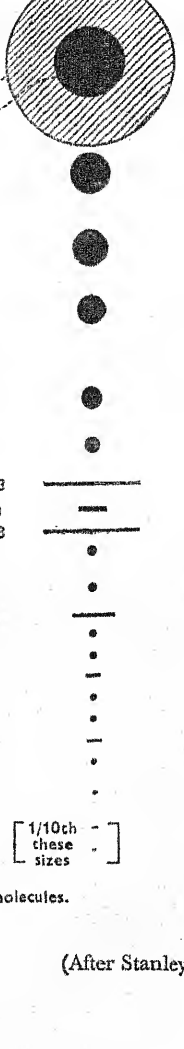
The one essential which the perfect parasite would retain is the power to transmit its own characters; most viruses "breed true" and can be propagated unchanged for long periods. If Laidlaw's theory were true we should then, as he suggests, be in sight of a biochemical study of the nucleo-proteins which transmit the characters of a species.

In stating the vitalistic theory of viruses we seem to have approached perilously near to Aristotle's indistinct and doubtful boundary which separates the living from the non-living.

The virus as a chemical substance.

We come now to consider the possibility that the viruses are of a chemical "non-living" nature, and here it must be stated the evidence, in the case of some viruses at all events, in favour of the non-vitalist theory is almost

	Molecular weight $\times 10^{-6}$ (Particle weight $\times 6{\cdot}06 \times 10^{17}$)	Diam. or length × width in mμ
Red blood cells	173 000 000	7500
Bacillus prodigiosus	173 000	750
Rickettsia	11 100	300
Psittacosis	8500	275
Vaccinia	2300	175
Myxoma	2300	
Canary pox	2300	
Pleuro-pneumonia organism	1400	150
Pseudo-rabies	1400	
Ectromelia	1400	
Herpes simplex	1400	
Rabies fixé	800	125
Borna disease	800	
Influenza	700	120
Vesicular stomatitis	410	100
Staphylococcus bacteriophage	300	90
Fowl plague	300	
C_{16} bacteriophage	173	75
Chicken tumour I	142	70
Tobacco mosaic	43	430 × 12·3
Cucumber mosaics 3 and 4	43	
Gene (Muller's est. of max. size)	33	125 × 20
Latent mosaic of potato	26	430 × 9·8
Rabbit papilloma (Shope)	25	40
Equine encephalitis	23	38
Megatherium bacteriophage	23	
Tobacco ring spot	13	182 × 10·4
Rift valley fever	11	30
Tomato bushy stunt	9	28
Haemocyanin molecule (Busycon)	6·7	59 × 13·2
Yellow Fever	4·3	22
Louping ill	2·8	19
Haemocyanin molecule (Octopus)	2·8	64 × 8
Poliomyelitis	0·7	12
Staphylococcus bacteriophage	0·4	10
Foot-and-mouth disease	0·4	
Haemoglobin molecule (Horse)	0·069	2·8 × 0·6
Egg albumin molecule	0·040	1·8 × 0·6



Comparative sizes of bacteria, viruses, and protein molecules.

Fig. 7

(After Stanley)

overwhelming. The idea of a "non-living" virus has been introduced in various forms, though essentially they all mean the same thing. Thus, viruses have been variously stated to be: an autocatalytic protein, a "loose" gene, a transmissible alteration of metabolism, a product of the disordered cell, a production of the chromosomes of the cell nucleus, a transmissible toxin when it causes the cells to disintegrate, and a transmissible stimulant when it causes the cells to proliferate.

It will be convenient perhaps first to consider the evidence in favour of the chemical nature of viruses, and after that to put forward one or two theories on the way a chemical virus might "reproduce" itself.

There is no doubt that Stanley, by his isolation from virus-diseased plants of a protein of heavy molecular weight with all the properties of the virus, has dealt a heavy blow to the vitalistic theory. This has been followed up by the work of Bawden and Pirie, who showed that the protein was a nucleo-protein, i.e. one containing phosphorus, and furthermore isolated a virus nucleo-protein in a true three-dimensional crystalline shape. Nevertheless, the criticism has been made that this protein has not been shown to be the virus itself and that the virus may merely be enclosed within the protein crystals. In Chapter II these objections were partially answered. The total evidence that virus and protein are one and the same has been admirably collated by Henderson Smith, and we quote freely from his article. If it can be shown that the protein is really pure, then it must be the virus itself; that there is no gross inhomo-

geneity or impurity is shown by the constancy of the protein obtained from such different sources as the phlox and tobacco plants. The infectivity of the protein is extremely high and it regularly infects a plant at dilutions of one in a hundred million or even of one in ten thousand million. Any procedure which removes or degrades the protein decreases the infectivity in a like manner. The temperature or degree of alkalinity and acidity which destroys the protein also destroys infectivity, and it has not been found possible to separate virus from protein by ultrafiltration through collodion membranes or by any other means. Again, if there is a second substance present it must have the same molecular weight because the protein gives a sharp sedimentation boundary in the ultracentrifuge which is characteristic of a pure protein. It must also have the same isoelectric point (the degree of acidity at which the particles have neither a positive nor a negative electric charge and are consequently precipitated out of solution), and in fact must have the same physical properties as the protein.

Another criticism by those upholding the vitalist theory of viruses is that the particles or aggregating units which form the crystals may be organisms. To this criticism there is also a definite answer. No known organism consists exclusively of protein. So far as we know at present every recognized organism contains diffusible constituents which can be leached out by appropriate means, but the virus proteins contain no diffusible constituents of any kind.

Again, in every known organism water is an integral part of its make-up, bound up with it and united in an intimate association. That is not the case with the virus protein, which does not unite with water. X-ray analysis has shown that water may penetrate between the particles, but it is a purely external relationship.

Further, Bernal has shown again by X-rays that within these particles the structure is regular and that the regularities persist at all concentrations. The scale of this regularity is of the order of 20 Å. (= 2 millimicrons), which is smaller than any particle of a living nature. If there was any more complex material present, any vital constituent within the particle, it could only be quite a small portion of the bulk of that particle. *In fact, there seems to be no ground for the view that the constituent particles are organisms as that word is ordinarily understood.*

Chemical nature of viruses and their power to multiply.

Having stated our case for the chemical nature of viruses we have next to propound some theory which will explain that property of viruses, so characteristic of life, the power to multiply.

Findlay has suggested that the virus may be somewhat similar in nature to certain enzymes such as proteinases. These are enzymes which have to do not only with the breaking down of protein molecules within the cell but also with the building up by synthesis, hydrolysis and replacement of complex protein molecules. There might therefore exist enzymes of this type which, when placed in the presence of a suitable host organism, would cause

the continuous production of protein by their power of synthesizing *replicas of their own structural pattern.*

Stanley draws a parallel between the multiplication of a virus within the cell and the behaviour of a saturated solution when a crystal of the same material is introduced into it. The atoms come out of solution and arrange themselves in an orderly manner after the pattern of the introduced crystal. It is conceivable that an autocatalytic protein might behave in a somewhat similar manner. A living cell presumably contains a large number of compounds such as salts, amino acids, carbohydrates and polypeptides, and complex materials such as proteins. From these compounds one might suppose that the virus protein, because of its characteristic structure and surface forces, is able to cause the necessary components to line up in the correct order as represented by the pattern of the intruding virus protein itself.

It is obvious that this could only happen provided that the cells of the host contained all the necessary component substances. If some of these were absent, then the virus would be unable to multiply and this would explain the extreme specificity of certain viruses for particular hosts or parts of hosts. Stanley points out that there is some evidence that tobacco-mosaic virus is built up from such component parts and not from the large proteins normally present in such cells, for this virus protein reproduces itself in both tobacco and phlox plants. It has been found by sensitive serological methods that the proteins normally existing in these

plants are quite different. This suggests, therefore, that the virus protein is built up from smaller serologically inactive units which must be common to both plants.

There has been much speculation of recent years on a possible connection between viruses and the Mendelian factor or gene. The gene which controls the phenomenon of Mendelian inheritance is conceived as a particulate structure within the chromosomes of the nucleus, and it possesses the power of self-duplication. It has been pointed out that several viruses have been proved to be nucleo-proteins, and the constant association of nucleo-proteins with the nucleus and the chromosomes suggests a possible connection between virus and chromosome. It is perhaps conceivable that the virus affects the genes and so starts the formation of virus protein.

If we accept as proved the chemical nature of some of the viruses we may ask whether it is not possible for some stimulus, other than the introduction into the cell of preformed virus, to start the production of virus protein. Since we are now speaking of a virus as a chemical substance, there is no need to invoke the phenomenon of heterogenesis (spontaneous generation) or to disturb the shade of Pasteur. Nevertheless, there is no evidence of such spontaneous production of viruses, although it would most easily explain certain facts such as the sudden appearance of foot-and-mouth disease in isolated districts, the universal infection of all King Edward potatoes with a virus which does not spread in nature,

and the constant appearance of tobacco-necrosis virus in the roots of plants.

To conclude this discussion on the nature of viruses we may appropriately quote the following extract from a recent paper by Stanley: "As we go from the admittedly non-living to the admittedly living, I think there must be a transition stage where there are entities that may possess some properties that are considered characteristic of non-living things. What could fill this place more simply and logically than the high molecular weight virus proteins that are intermediate in complexity between the protein enzymes and hormones, the wonderful properties of which we already recognize, and the system of proteins that we call protoplasm and which constitutes life. There is evidence that even within the virus group there is a gradual increase in complexity of structure from the small nucleo-proteins to the more elaborate elementary-body type of virus. There is, however, no sharp break despite the fact that in certain respects the structure of the latter may resemble that of a cell-type organism as much as it resembles that of the smaller viruses."

PART II

The Virus in Action

CHAPTER IV

HOW VIRUSES GET ABOUT

Infection by contact.

It is probably true to say that all virus diseases are infectious and that in every case the virus agent is capable of being transferred by some means or other from a diseased host to a healthy susceptible one. The methods of spread of viruses, however, are of several kinds, and the study of these methods is one of the most interesting and important chapters in the history of virus research. We have already seen how Ivanovski was able to infect a healthy tobacco plant with the mosaic disease by rubbing the leaves with the filtered extract of a diseased plant; this method of transmission is largely used in the study of certain plant viruses and is known as *inoculation.*

From earliest times it has been realized that a disease like smallpox was infectious, and we know that such virus affections as rabies, dog distemper, fowl-pox and many others are spread about easily enough. For convenience of discussion we may loosely classify the methods of dissemination of viruses and so divide these various agents into groups according to the way they get about. It must be realized, however, that some viruses can spread in several ways and so may appear in more than one group; in addition, they differ considerably in the degree of infectiousness.

In the case of certain viruses it is only necessary for

the diseased host to come into contact with the healthy susceptible host for the virus to be transferred. Into this category fall the most infectious types. In carrying out research on these very infectious viruses, the reader will understand that it is essential to guard against accidental spread or contamination which would not only vitiate the results of the research but might also start an epidemic or epizootic outside the confines of the research station. In carrying out research upon such extremely infectious viruses as those of dog distemper and foot-and-mouth disease the most elaborate precautions are necessary, and the following account, taken from Dunkin and Laidlaw's description of the former, gives some idea what these are.

The experimental house is a fly-proof structure divided into symmetrical halves. Each half is divided into cubicles completely separated from each other by wooden partitions. The walls and roof of this building are lined with uralite and the joints sealed as completely as possible. The cubicles measure $7\frac{1}{2}$ by $5\frac{1}{2}$ feet and are ranged round the outer walls, leaving a control space which contains a large tank of lysol for disinfection purposes. The two separate working sections are a great convenience, as the arrangement allows of one-half to be shut down completely while a thorough disinfection is in progress, and further, admits of a stricter separation between experimental animals in special cages. Entrance to either half of the building is only possible through a short corridor, the floor of which is sunk and constantly contains 3 inches of 1% lysol. Every person entering

the building wears rubber boots to thigh or knee and a rubber-covered overcoat which overlaps the boots. On entry into the building boots and coats are swilled down with 1% lysol. Rubber gloves are worn by the attendants and they also are disinfected with lysol. After every visit to a cubicle containing an animal, whether showing symptoms or apparently normal, the rubber armour is washed down with lysol once more, and hands and gloves are disinfected. The floors of the central halls are thus kept wet with disinfectant all the time work is in progress, and any infective material settling on coats and floors is rapidly sterilized. No two cubicles are opened at one and the same time. In addition to these precautions a special type of cage is used for each experimental animal, while a separate building, distant about 50 yards from the laboratory and experimental house, is set apart for the breeding stock.

Very similar precautions are taken in dealing with foot-and-mouth disease, and in addition the whole research station is made rat-proof by being fenced round with a strong close-mesh netting rising to a height of 6 feet above ground and buried 2½ feet below ground. An extra semicircular cowl, projecting outwards about a foot, is fixed to the ridge of the fence to prevent the rats climbing the fence.

After an outbreak of foot-and-mouth disease has been reported on a farm, the most elaborate precautions are taken to prevent the spread of the virus. All affected animals and those in contact with them are slaughtered and their carcasses burned. The farm buildings are

sprayed with disinfectant, and any heavily contaminated material is burned while the clothing of attendants is fumigated. Manure is dealt with by close packing, so that the heat of fermentation destroys the virus except on the surface, which is sprayed. The disinfectants now used are lysol and alkalies such as washing soda (sodium carbonate) or dilute caustic soda.

In our category of viruses, grouped by their mode of dispersal, we have put in the first group those which spread by "contact". This method of spread is not, however, quite so simple as it appears; in other words the exact mode of entry of the virus into the host is not yet understood in every instance. Thus in the case of the very infectious virus of foot-and-mouth disease the precise method of transference of the virus and the conditions requisite for its entrance into the tissues have not been fully demonstrated. The report of the Foot-and-Mouth Disease Committee, issued in 1937, states that it is probable that transmission most readily occurs when a bovine with recently broken vesicles in the mouth and other air passages is near enough to other cattle to infect the air breathed with a spray of minute droplets of buccal or nasal secretion.

The question which the intelligent reader will now ask is, Can the virus pass through the unbroken skin or epidermis of the host? All the viruses in the "contact" group can be spread to healthy susceptible hosts by injection beneath the epidermis, but with one or two exceptions this method does not apply in nature to the "contact" group of viruses.

Perhaps the best example of a natural injection method of virus spread is given by rabies. In nature there is only one mode of spread of this virus and that is through the bite of an infected animal, by which infective saliva is introduced subcutaneously into the bitten animal. It seems that contact of the virus with susceptible nervous tissue is a necessary occurrence for infection. It is generally assumed that plant viruses require a wound, however minute, the breaking of a hair suffices, for entry into the plant. Ivanovski transferred his tobacco-mosaic virus to a healthy plant by rubbing it into the leaves and thereby breaking the surface hairs. This method is used to transmit a number of plant viruses, but many, as we shall see later, cannot be transferred so simply.

The virus of yellow fever is transmitted in a special way in nature and does not spread by contact, but it can be transferred experimentally to monkeys by rubbing the skin with a glass rod dipped in virus. Another virus belonging to our "contact group" is that of "fowl-pox", an eruptive disease which attacks the pigeon and domestic fowl. Contact between diseased and healthy birds is essential for infection, since the usual method of propagation is probably through wound infection. Application of the virus to the *unbroken* comb, skin or buccal mucosa fails to infect, but small wounds frequently result from the pecking and fighting inseparable from a flock of fowls. In a simple experiment Burnet has shown that pigeons do not become infected when virus is added to the drinking water even for so long a

period as one month unless sharp grit, liable to cause small wounds, is fed to the birds at the same time.

It is possible to find an interesting parallel to this experiment in the virus infection of plants. The virus of tobacco necrosis is confined to the roots of affected tobacco and other plants, and the mechanism of infection is interesting and unusual. It is thought that the virus reaches the soil by methods which will be discussed later, and once in the soil is washed down towards the roots. As the roots make their way through the earth the delicate root hairs become ruptured by contact with the sharp particles of grit, etc., in the soil, and opportunity is thus afforded for the virus to enter the cells. If, however, the plants are grown in a medium (such as a liquid nutrient) which cannot break the root hairs or abrade the root epidermis, then the virus is unable to enter the root *unless* it is deliberately injured.

There is a certain virus affecting the potato plant which is so common and so universally distributed among the potato crops of the world, that in the U.S.A. it is popularly known as the "healthy potato virus". For many years workers have endeavoured to find out how this virus is passed from plant to plant in the field, and the latest theory is that the virus passes by contact between diseased and healthy plants when the wind lashes one against the other. An experiment was designed to test this theory: An infected potato plant was placed, in a clean empty glasshouse, in contact with a number of virus-free potato plants, each one in contact with the other. An electric fan was adjusted so as to

create a draught of air comparable to a breeze blowing at 6 miles an hour. This air current set the leaves of the potato plants rubbing against each other, and after a period to allow for the development of the disease it was found that the virus had spread to eight out of twenty-seven plants. While it is clear that this is one method of dispersal of the potato virus, it is not yet proved, however, that no other means of spread exists.

Perhaps the best example of a plant virus which spreads by contact is afforded by the virus of tobacco mosaic, the most infectious plant virus known. Dispersal in this case is entirely by contact, either by an infected tobacco plant rubbing against its neighbour or by the transference of infective material on the hands and implements of workers. It is necessary, however, even for this very infectious virus, that a hair should be broken or other minute wound made to allow entry into the plant. The following story illustrates the infectious nature and power of resistance of the tobacco-mosaic virus. A tobacco grower in America had noticed that his crop always contained an unusually high percentage of mosaic-diseased plants as compared with his neighbour's crop; he could not understand the reason. Ultimately, however, he noticed that several of his workmen were in the habit of chewing tobacco, and investigation showed that such tobacco frequently contains the virus in an infectious state. The reason for the high percentage of mosaic-diseased plants then became clear, the workmen were themselves infecting the plants either by indiscriminate spitting or by traces of tobacco

juice on their hands. It has now been found an economic proposition to issue the workers with sterilized chewing tobacco. It is also a fact that many commercial brands of cigarettes and pipe tobacco, which are not subjected to high temperatures in the curing process, contain active tobacco-mosaic virus.

We have seen, then, that the more infectious viruses spread by contact, when the diseased host, or infective material from it, rubs or touches a healthy susceptible host in such a manner that the virus enters a living cell. We come now to consider the question as to whether a virus can travel in the air, even if only a short distance, and enter and infect a healthy susceptible organism.

Infection in the air.

The possibility of air-borne virus infection depends largely on two conditions: first, the ability of the virus to retain its infective power for a sufficiently long period of time after it leaves the first host, and secondly, its ability to enter and infect a second host without further assistance. In order to retain infective power for long periods while floating in the air a virus must be fairly stable and must be able to withstand a certain amount of desiccation; it must also be resistant to oxidation. Other less resistant viruses may retain infective power for an hour or less while they remain suspended in the air in droplets of moisture. The virus of epidemic influenza is one of this latter type, as is shown by some recent experiments in America. The virus was atomized into the air of a steel chamber of 200 cubic feet capacity

and then collected at intervals by means of an air centrifuge. It was found that the virus retained its infective power under these conditions for periods within 1 hour of suspension: but after this time no active virus could be obtained from the chamber.

There has recently been discovered an interesting plant virus which is a good example of an air-borne infection. The virus itself, known as tobacco-necrosis virus, is interesting and unusual in that it occurs in the roots only of a variety of different kinds of plants without causing any apparent disease with the exception of occasional symptoms in the two first leaves of the tobacco plant. It was this occasional development of symptoms which first led to the isolation of the virus, as otherwise its presence would never have been suspected. Now, when a new virus disease is discovered, the first thing to be done is to prevent its spreading: and that cannot be achieved until one knows how it spreads.

The following experiments will illustrate how one possibility after another was eliminated until the problem was solved. The whole problem can easily be translated into terms of a detective story where each potential suspect is considered until the evidence points conclusively to the guilty person. In our scientific detective story then, the potential suspects are all the known methods of spread of plant viruses with the possibility of some new method in the background. The known methods of spread of plant viruses are, first, as we have already seen, contact of diseased and healthy plants; secondly, by the agency of insects, an interesting re-

lationship which we shall consider presently; and thirdly, transmission in the seed of infected plants. We do not include transmission by grafting in our list because that is a purely artificial method of spreading a virus.

Before we proceed to the actual detective work it is necessary to explain briefly how the presence of the virus in the roots is detected. Unfortunately the virus worker, unlike the chemist, cannot make his identification by means of a chemical reaction in a test-tube but has to rely on other methods. In dealing with most animal viruses, workers usually have some small animals which are easily reared, such as guinea-pigs, rabbits, rats, mice or ferrets, upon which they can make a test for the presence of the virus. The reactions of these animals to many viruses are generally rapid and easy to recognize, e.g. the production of lesions on the skin or other specific symptoms. The procedure of the plant-virus worker is on the same principle; his "laboratory animal" is a particular plant species which reacts in a specific and characteristic manner to inoculation with the virus in question, and by this specific reaction identifies just that virus and no other. Such plants are known as "differential hosts" or "indicator plants". In the case of the virus with which we are at the moment concerned, the differential host is the French bean, and when the roots of the plant to be tested are ground up in a mortar and rubbed on to the bean leaves, the presence of the virus in the roots is indicated by the rapid development on these leaves of numerous circular red spots or lesions.

To return now to our detective work, we have first to ensure that no insects are present which might be concerned in the spread of the virus. This is achieved by growing the experimental plants in an insect-proof glasshouse which has all openings to the exterior, such as ventilators, covered in with a fine gauze made of phosphor bronze and through which insects cannot penetrate. For further precautions the house is fumigated at regular intervals and the soil in which the experimental plants are to be grown is heated in an autoclave whereby the temperature of the soil is raised under pressure to 120° C.

Having thus eliminated the insect from our list of suspects we have next to consider the question of transmission by contact. Since this virus is located only in the roots, the possibility of contact is easily avoided by growing each tobacco plant in a separate pot. We come now to our third possibility, that of transmission in the seed. This is tested by growing large numbers of tobacco seedlings on damp blotting paper contained in flat glass dishes fitted with covers. By grinding up samples of these seedlings at intervals and inoculating them to French beans, the virus could be detected if present in the seedlings. Having satisfied ourselves by this preliminary test that the virus is not carried in the seed, we can proceed to our first experiment and the tobacco seed is sown in the sterilized soil contained in isolated pots standing in the insect-proof glasshouse. It should be mentioned that an additional precaution is necessary; before sowing, the seed is sterilized externally by im-

mersion in a weak solution of corrosive sublimate. The plants are now allowed to grow normally and are subject to the usual routine of glasshouse culture. When the plants are from 5 to 6 weeks old, samples are taken and the roots are tested for virus by inoculation to French beans.

In spite of these precautions the virus is still found in the roots of tobacco plants growing under the conditions we have described. We have therefore to look for other possible means of spread for the virus, and there are left only two, the water and the air. Before testing out these two possibilities, we must consider whether the plants themselves may be producing the virus. This may appear a fantastic theory, but the reader will see on p. 42 that it is not so impossible as it seems. Now to test whether the plant is itself giving rise to this virus we must somehow grow our plants under conditions in which we *know* the plant to be the only living organism and in which the entry of any organisms from the outside is impossible. This method of growing plants is known as "sterile culture" and is carried out as follows. Some large glass flasks are partly filled with soil and their necks firmly plugged with cotton-wool. Through the cotton-wool plug runs a narrow glass tube, itself plugged at its outer end with cotton-wool, and reaching down inside the flask just clear of the cotton-wool in the neck. The flasks are now placed, just as they are, in the autoclave and heated for 2 hours under pressure at a temperature of 120° C. This process destroys all living organisms in the flask and its contents and renders them

sterile. The next process is to sterilize the outside of the tobacco seeds, and to accomplish this the seeds are washed several times in a weak solution of corrosive sublimate. A few of these sterilized seeds are then dropped through the narrow tube in the neck of the flask and the cotton-wool plug replaced. The flasks are placed on one side and the seeds allowed to germinate. In one such experiment sixty plants grown under these sterile conditions were tested for virus in the roots with negative results.

We may therefore assume that the plant is not itself producing the virus, and we must proceed to test the other two possibilities of transmission by water or air. In order to find out if the virus is water-borne the first experiment of growing the tobacco plants in isolated pots of sterilized soil is repeated with the additional precaution that all the plants are watered with tap water that has been boiled. The results of this experiment show at once that we are on the right track; the amount of virus infection under these conditions falls tremendously, but it is not quite eliminated. Investigation of the water tanks from which the plants were watered in the previous experiment revealed the presence of the virus in the sludge at the bottom of the tank, whence it was scooped into the watering can and so poured on to the soil. Our piece of detective work is now practically accomplished; the virus can be transmitted in the process of watering the plants, but since some plants still contained virus, even when boiled tap water was used for watering, we must next test the

possibility of an air-borne infection. The first thing to do is to find out if the virus is actually in the air of the glasshouse. To do this, a simple suction apparatus is set up whereby the air of the glasshouse is drawn through a small quantity of water contained in each of six gas-washing bottles. The water in the bottles is tested at intervals for virus by inoculation to the leaves of French beans.

Such an experiment has given positive results, and virus was eventually found in the water contained in all six of the bottles. So far then we have proved that the virus is in the air, and we have considerable circumstantial evidence that infection of the plant can take place by this means. In order to prove our case, however, it is necessary to demonstrate the actual contamination of plants by virus from the air under controlled conditions. We enter now upon the last phase of our investigation, and it is planned in the following way. Two small chambers measuring about 5 by 4 feet are built inside the insect-proof glasshouse; these chambers are mostly of glass, but each is provided with a wooden front in which is cut a circular hole large enough to admit a potted plant. A wooden disk is fitted to slide over the hole, closing it completely. In the panel there is also cut a second small hole, and this is covered with a square of thick rubber sheeting, through which passes a tightly fitting metal tube, long enough to reach to the farthest corner of the chamber. Inside, each chamber is lined with zinc and every effort is made to reduce the circulation of air between the chambers and the main glass-

house. By means of the metal tube fixed in the rubber sheet it is possible to water the plants without opening the chambers. Pots containing sterile soil and tobacco seeds, sterilized externally, are then introduced through the "porthole" in the front of each chamber. We have now a series of about 120 tobacco plants growing in two chambers, sixty in each, under identical conditions and with the minimum possible communication with the surrounding air of the main glasshouse. A third set of tobacco plants is prepared under similar conditions except that they are not enclosed in a chamber but are growing free in the glasshouse. These three sets of plants are all watered only with boiled tap water.

By the foregoing precautions, then, we have eliminated so far as is practicable all possible modes of contamination with the virus and we are ready to start the experiment. When the seedlings are about 2 inches across, a suspension of virus is atomized into the air above the plants growing in one chamber. The plants in the other chamber and those outside are not touched, and these two sets of plants act as checks or "controls". At intervals of a week or 10 days, the virus is again lightly sprayed into the air of the experimental chamber. Tests for the presence of the virus are made at weekly intervals by grinding up the roots of two or three plants from each of the three sets and inoculating them to our test plant, the French bean. Here then are the results of one such experiment: About 3 weeks after the commencement of the test, the inoculations from the roots of the plants in the experimental chamber began to show

the presence of the virus. Thereafter in all the subsequent tests, fifteen in number, virus was found to be present in the roots of those plants growing in the chamber in which virus had been atomized. The roots of the plants in the other chamber and of those growing free in the glasshouse contained no virus. Here then is the answer to our problem: the virus is not spread by insects nor by contact, neither is it carried in the seed, but it is transmitted in water and can be air-borne. By these last two methods it reaches the soil and thence enters the roots of any susceptible plant growing in the soil.

In the important work of Dunkin and Laidlaw on the virus disease known as dog distemper we can find a parallel to our experiments with the air-borne plant virus. In their experiments two ferrets were put in separate Topley cages, a special type of cage used in this work, and these were placed in one cubicle of the experimental house. The distance between the cages was approximately 2 feet. One ferret was infected by injecting hypodermically a small quantity of blood from a sick animal, the second ferret was kept untreated as a control. The inoculated ferret sickened on the 10th day (the normal incubation period for a ferret), and the control animal remained healthy. The inoculated ferret became rapidly worse and was killed, when moribund, on the 4th day of the disease. The control ferret remained healthy until the 20th day of the experiment, when it sickened and subsequently developed typical distemper symptoms. This result was quite unexpected, and it was repeated three times in succession with the same result.

Exactly 10 days after the inoculated ferret showed its first symptoms the control animal developed the disease; and this although the control animal was shut up in a Topley cage, never handled and the full antiseptic ritual scrupulously observed. Dunkin and Laidlaw have found that this experiment has been confirmed unintentionally several times; in fact, so regular has been the infection that separate cubicles must be reserved for every experiment unless the result can be secured in less than 20 days.

There seem to be only four possible paths of infection worthy of consideration. Infection by food is regarded as unlikely in view of the precautions taken in regard to feeding and also because of the regular time interval between the first symptoms in the inoculated ferret and the onset of disease in the control; further, control animals kept in Topley cages but in separate cubicles do not develop the disease, although fed in precisely the same way. Parasites which might serve to spread the virus have never been observed on ferrets. The experimental house is fly-proof and flies have very seldom been seen inside it. One is forced therefore to the conclusion that the only possible explanation of the spread is that the virus is air-borne over short distances. This view is further supported by other experiments of a different nature. The disease may be passed from dog to ferret and ferret to dog without actual contact occurring. It is usually sufficient to place a ferret within a cage in a room with a dog suffering from distemper and in 10 days' time the ferret will develop the disease.

Infection carried by insects.

So far then, we have examined those methods of dissemination of viruses (by contact, in the air, in water) in which no other living agent is required to assist the passage of the virus from the diseased to the healthy organism. We come now to consider the most interesting method of virus dispersal in which the infective agent is dependent upon an insect for its dissemination. This relationship between the virus and the insect which spreads it is a curious and interesting one, and much work remains to be done before the relationship is fully understood. In considering this aspect of the subject the distinction between an insect *transmitting* a virus and an insect affected by a *virus disease* of its own (see Chapter VII) must be clearly understood.

The reader is probably familiar with the classical work of Manson and Ronald Ross in which it was demonstrated that the malarial parasite is dependent upon the *Anopheles* mosquito for its transfer from host to host. While we may have occasion to quote this as a comparison with the insect transmission of viruses, it is not, as we shall see, a complete analogy. In the case of the malarial parasite and the mosquito we have an undoubted organism which passes a portion of its developmental life-cycle within the insect's body; where insect and virus are concerned, although some kind of relationship between the two is suspected, we are by no means certain of the nature of this relationship.

One of the earliest records of an association between

a virus and an insect was published in 1901 and showed that the virus causing mosaic or "stunt" disease of rice is carried from plant to plant by a kind of sap-sucking insect known as a leaf-hopper.

It must not be supposed that viruses are transmitted by any casual insect which happens to have fed first upon a virus-diseased host before it feeds upon a healthy one. On the contrary, just as malaria is not carried by every kind of mosquito, so it usually requires a particular insect or type of insect to transmit a virus. If we examine the insects which have been found responsible for transmitting the viruses which affect plants, we notice at once that they nearly all belong to one group and, moreover, all obtain their food in the same manner. These insects are known as "vectors", and they do not feed upon the actual tissues of plants but extract the sap by means of a long sucking beak which is inserted into the plant. In fact, they feed in much the same way as the mosquito, but extracting the sap of the plant instead of the blood of an animal.

The common green-fly or aphis which feeds upon the rose may be taken as an example of this type, and indeed the aphides are among the most efficient vectors of plant viruses. The feeding apparatus of this kind of insect consists of a beak or rostrum in which slide two pairs of thin needle-like stylets. Two of these stylets have each a pair of grooves on their inner faces, and these grooves when in contact form two very fine canals. When the insect feeds, it presses the rostrum on the surface of the leaf, the rostrum bends, becomes foreshortened and the stylets

are pressed into the tissue. Saliva is then forced down one of the canals into the plant and, together with a quantity of sap, is sucked again up the other canal and so into the body of the insect. It is by this mechanism that a healthy plant is thought to become infected by a virus. The insect feeds upon a virus-diseased plant and while sucking the sap also perforce imbibes some of the virus in that sap; later it may move to a healthy susceptible plant, and in feeding again it injects along with its saliva some of the virus picked up when feeding on the previous plant (see Figs. 8 and 9).

The number of plant viruses which depend upon insects for their transfer from plant to plant is very large, while the animal viruses similarly transmitted are less numerous but still of great significance. Aphides or green-fly are responsible for the spread of many plant viruses, and one species, known as the peach or spinach aphis, is capable of transmitting no less than twenty-one separate viruses. A slightly different type of insect, the leaf-hopper, is also much concerned with plant viruses and these insects spread certain viruses affecting sugar-beet and maize which are of supreme economic importance. Many people are familiar with the "greenhouse white-fly" in this country, a very small moth-like creature which, in spite of its rather different appearance, is a sap-sucking insect closely related to the aphis. A particular species of "white-fly" transmits a devastating virus disease of cotton which threatened a few years ago to wipe out the whole of the cotton crop in the Sudan. That very tiny insect known as a thrips is the vector of

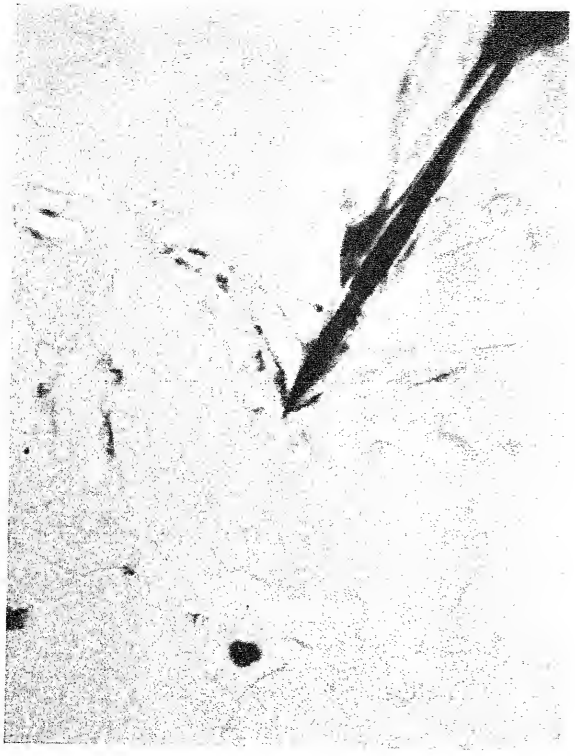


Fig. 8. Photomicrograph of the beak of a sucking insect in the act of penetrating a leaf: note the epidermis of the leaf giving way before the pressure of the insect's stylets.

Fig. 9. A similar photograph to that reproduced in Fig. 8: note the drop of saliva at the end of the stylets. It is this type of insect which transmits plant viruses which are carried in the saliva.

an important virus affecting tomatoes and many ornamental plants.

Of the viruses affecting animals including man there is a group causing rather similar diseases, all of which are spread by blood-sucking flies. The well-known virus disease of yellow fever is spread by one or more species of mosquitoes. The somewhat similar but milder disease, dengue fever, is carried by the same species of mosquito. A mild fever, known on the Continent as "summer influenza", is transmitted by a slightly different type of fly, *Phlebotomus pappataci*, popularly called the sandfly and hence "sandfly fever". Horses are susceptible to a virus disease of the "sleepy sickness" type known as "equine encephalomyelitis", the virus causing which is also spread by a mosquito. Sheep in Scotland are liable to attack by "louping ill", which is not transmitted by an insect but by an allied arthropod, a species of tick. There is an important group of diseases affecting man known as "Rickettsia" diseases. It is a disputed point whether these disorders should or should not be considered as "virus diseases". The causal agent, named after its discoverer Ricketts, is a minute body considerably smaller than a bacterium which is, however, visible under the highest power of the microscope. We may perhaps regard these disease agents as intermediate between the bacteria and the true viruses, and like viruses they cannot be cultivated on artificial media. Probably the best known Rickettsia diseases are typhus fever and its mild relation, trench fever, both of which were prevalent on various fronts in the Great War. These two

diseases are spread by a blood-sucking insect, the body louse, *Pediculus vestimentis*. The Rickettsia which causes a typhus-like disease, called Rocky Mountain spotted fever, is carried by the wood-tick, *Dermacentor venustus*; here curiously enough man only, out of the many animals parasitized by this tick, is susceptible to the disease. Tsutsugamushi, the last of the Rickettsia diseases, is spread by a mite, *Trombicula akamushi*, of which only the larval stage is capable of transmitting the disease. The interesting relationships between these various diseases and the insects and other arthropods which transmit them are discussed in some detail in the next chapter.

Other means of infection.

The title to this chapter is "How viruses get about", and we have briefly described the methods of spread of several of these infectious-disease agents. The problem, however, is by no means solved, since there are many viruses of which the mode of dissemination is unknown or merely speculative. So far as the plant viruses are concerned, there are between fifty and sixty which spread by unknown means. For example, a very great deal of research work has been carried out in India in an endeavour to find out by what means the virus causing the "spike disease" of sandal is spread. At the moment the evidence seems to suggest that one of a number of nocturnal insects is the vector.

A certain virus affecting potatoes presents another baffling problem; here we have a virus which does not spread at all but it is present in all potatoes of that well-

known English variety, King Edward. This virus has apparently no insect vector and cannot be transmitted by mechanical means but only by grafting. It must therefore have been present in the original King Edward seedling whence it has been perpetuated in the tubers of this variety all over the world. The interesting points are, how did the original King Edward seedling get infected and where did the virus come from?

Similar uncertainties exist concerning the means of spread of some of the most important animal viruses. In foot-and-mouth disease there appears to be no material connection between one outbreak and another, and many suggestions have been made to account for outbreaks which suddenly occur in isolated districts. As regards the possibility that insects transmit the virus, experimental evidence does not lend support to the view, since it seems that the common house-fly, the blood-sucking stable-fly and the bed-bug (*Cimex*) are not responsible. There are of course other blood-sucking insects and related arthropods which do not appear to have been tested, but against them all is the fact that the disease seems to spread in winter when insects are absent. Another suggestion which is supported by a certain amount of circumstantial evidence is the possibility that the virus is carried mechanically from place to place on the feet of birds, especially starlings—mechanically carried because birds are mostly resistant to the disease itself. In support of this suggestion we have the following facts: Starlings migrate in large numbers from the Continent where the disease is ram-

pant, and it seems that outbreaks generally start near the coast. Moreover, sudden outbreaks do not occur in Scotland where the migratory starlings do not go. It would seem to be worth while to inaugurate some experiments to test the possibility whether birds really do carry the virus, e.g. trapping the migratory starlings and testing them for the presence of virus. The possibility of wind-borne infection must also be mentioned, although outbreaks of the disease are known to spread against the prevailing winds. The suggestion has also been made that the virus may be air-borne in yeasts which would enable the virus to retain its infective power and so to travel long distances; but it is doubtful whether the association between yeast and virus would really enable the latter to remain infective for long periods.

If we reject all these suggestions as to the method of spread of the virus causing foot-and-mouth disease, then we must fall back upon two possibilities—one that there is some common animal (other than the cow, pig, sheep and goat which are all normally susceptible) which acts as a source of infection and the other possibility is that the disease starts afresh from virus already present but lying latent in the cow. Careful search has been made for some common animal in which the virus might be universally dispersed, and it has been shown that both the rabbit and the rat are experimentally susceptible to infection. A more significant discovery, however, shows that the hedgehog is not only susceptible and can be found naturally infected but that infection can be conveyed between cow and hedgehog. In country districts

there is frequently a tendency to regard the hedgehog as rather a sinister animal and at the end of the eighteenth and the beginning of the nineteenth century it was the custom of churchwardens in Hertfordshire to put as high a price (*4d.*) on the head of a hedgehog as on that of a polecat. "Urchins" were supposed to do something to cows which reduced the milk supply, and from this rose a belief, still held in many country districts, that the hedgehog sucked the udders of cows when the animals were lying down. Here again, however, it is difficult to correlate outbreaks of foot-and-mouth disease with the presence of hedgehogs, although the following facts do suggest the possibility that these animals may have some connection with the spread of the virus. It has been observed that hedgehogs are liable to serious mortality in the winter months when outbreaks of foot-and-mouth disease are prevalent, that they are heavily parasitized by ticks which are also capable of biting domestic stock, and that they do sometimes hibernate in cowsheds. Against these facts it must be stated that outbreaks of foot-and-mouth disease occur in the apparent absence of hedgehogs, and furthermore, Galloway has shown that the tick in question is incapable of harbouring the virus for long, and it is doubtful if the tick can even transmit the virus at all.

The second of our two remaining possibilities was that the virus lies latent in the cow and is then awakened to activity by some stimulus or by some particular set of environmental conditions. By a survey of other viruses, circumstantial evidence can be found to support this

hypothesis, for instance, the virus disease known as labial herpes seems to have no connection with any spread from person to person but has a definite connection with other diseases and conditions which appear to stimulate it to activity. Thus, everyone is familiar with the person who habitually develops herpes on the lips when suffering from a bad cold, and the association of herpes with severe catarrh is common enough to give rise to the name for herpes of "cold sore". Again, there is the peculiar phenomenon of "Virus III" which comes to light when inoculation is made from the testes of apparently normal rabbits to the testes of other apparently normal rabbits. The explanation which is most probable is that the virus is present in small quantities in the testis and this passage through several rabbits seems to increase the virus to a point where its effects become obvious and an inflammatory condition is produced.

Before completing this account of the way viruses get about mention must be made of the propagation of plant viruses in the reproductive parts of infected plants. Since plant viruses are mostly systemic in their hosts, which means that the infective agent is distributed all over the plant, then it follows that the virus is present in all those portions, tubers, bulbs and cuttings, which are used in vegetative reproduction. Since so many plants are multiplied in this way, virus infection becomes doubly important, for not only is the plant rendered useless but it is impossible to take cuttings or propagate from it vegetatively in any other manner.

This is well illustrated by the rapid deterioration of potato crops in England if the grower saves his own "seed" for a number of years. If the crop is grown in an area favourable to the spread of virus disease, each year shows a progressive increase in the amount of virus infection with a corresponding decrease in the quality and yield in the crop. Infected tubers and bulbs are also instrumental in spreading virus diseases, introducing them into new localities and even new countries. That serious virus disease of tomatoes, spotted wilt, was first described in Australia in 1915; now its distribution is almost world-wide, and one can hardly doubt that it has been transported into different countries in the bulbs and tubers of various kinds of infected plants.

So far we have mentioned only the vegetative organs of reproduction, and it is rather a curious fact that plant viruses are seldom transmitted through the true seed. There are, it is true, several authentic cases of such transmission, the best known being the virus of bean mosaic which is transmitted through the seed of the French bean, *Phaseolus vulgaris*. In consequence, this virus is present wherever the bean is grown.

CHAPTER V

THE RELATIONSHIP BETWEEN VIRUSES AND THE INSECTS WHICH SPREAD THEM

Does the virus undergo some change in the insect's body?

In the previous chapter we have mentioned malaria, and the mosquito which transmits it, as a partial analogy with virus and insect. In the case of malaria, however, there is an undoubted organism undergoing a necessary phase of its life-cycle within the body of the insect; the mosquito is the nurse of the malaria organism, so to speak. Moreover, it is possible, with the aid of the microscope, to see the stages of this organism within the insect.

With the virus, however, we have no such evidence. As we cannot see it in the insect's body we can only draw certain deductions from the joint behaviour of insect and virus. We have seen that some plant viruses cannot be transferred from a diseased to a healthy plant merely by injecting virus sap from the former into the latter. They can only be spread by the medium of a particular kind of insect, in some cases by one insect species alone. Moreover, with this type of transmission there is usually a period of delay after the insect has fed on the virus-diseased plant before it can transmit the virus to a healthy plant. Does this mean that during the period of delay in the insect the virus is undergoing some kind of

change within the insect's body which allows it to multiply in the new plant? It may be so, but at present we have no knowledge of what this supposed alteration may be or in what way the insect attunes the virus for its entry into the fresh host. It is, however, permissible to speculate on the relationship between insect and virus and on what happens to the virus when it is swallowed by the insect.

Virus and leaf-hopper.

There is an important virus disease of asters in the U.S.A. known as "aster yellows", so called because of the yellow colour in the leaves of affected plants. This virus is transmitted by a leaf-hopper, *Cicadula sexnotata*, and some interesting experiments have been performed with this insect and the virus. Leaf-hoppers which have fed upon a "yellowed" aster plant are not able to infect a healthy aster plant with the virus until after a period of 9 days from the time of feeding on the diseased plant. The consequence of this is that the insect cannot become a vector in its larval or "nymphal" stages because those stages are shorter than the 9 days mentioned above. If, however, the development of the insect is artificially delayed by subjecting the insect to low temperatures, then it becomes able to transmit the virus whilst still a nymph.

Now if we compare this phenomenon with the transmission of yellow fever virus by the mosquito, *Aëdes aegypti*, we find a slightly different state of affairs. In the mosquito also there is a delay in the development of

infective power which at a temperature of 28° C. [82·4° F.] is from 9 to 10 days. In this case, however, unlike that of the leaf-hopper and the plant virus, low temperatures seem to affect the transmitting power of the mosquito. For example, a mosquito which has ingested virus does not become infective at a temperature of 10–15° C. [50–59° F.], but will become so if, after several weeks, the temperature is raised to 28° C. On the other hand, a mosquito that has become infective retains its power to infect even if kept at a temperature as low as 10–15° C. It may become infective if kept at 18° C. [64·4° F.], but the delay is very prolonged.

How can we explain this "incubation period" of the virus in the insect and the difference in the behaviour of these two insects in regard to the viruses they transmit? It can be explained readily enough so far as the mosquito is concerned if we assume that the virus must multiply inside the insect before there is sufficient to form an infective dose. It is certainly true that the rate of multiplication of a virus inside an organism is affected by the latter's metabolism. For instance, it is very difficult to infect an old and mature plant with a virus, and it has been observed that a hedgehog infected with the virus of foot-and-mouth disease just prior to or during its hibernation period does not develop the typical lesions of the disease until the following spring. In these cases, however, we are dealing with *diseases*, and there is no reason for assuming that either the mosquito or the leaf-hopper are in any way affected by the viruses they transmit. In the case of mosquito-borne viruses, however,

there is some evidence of multiplication of virus inside the insect. Thus, the "western" strain of equine encephalomyelitis virus has been carried through a series of fifteen lots of mosquitoes, *Aëdes aegypti*, the same species as that transmitting yellow fever. This was done by feeding normal mosquitoes on the crushed bodies of infected ones, thereby reaching a dilution of virus far beyond that which could infect if it had not increased in the body of the mosquito. This experiment was discontinued after the seventeenth passage, when all the virus could still be demonstrated in undiminished quantity. Again it has been shown that after the mosquito has ingested blood containing the virus of yellow fever, the virus content within the insect's body falls sharply for a day or two and then commences to rise again until it reaches a concentration higher than that shown by the insect immediately after feeding on its source of infection.

It is not so easy to explain the delay in infective power, in the case of insects transmitting plant viruses, on the ground of necessary multiplication of virus. Thus, an aphis which transmits one of the commonest potato viruses rapidly loses its power to infect healthy plants and must have recourse to a fresh supply of virus before it can infect again. It is possible in this instance that the virus in question is digested by the enzymes in the insects' juices and thus is soon destroyed. Again, if we test the transmitting power of the leaf-hopper which spreads the virus of sugar beet "curly top", we find that there is a gradual drop in the percentage of healthy plants in-

fected by the insect, in a series of day-to-day transfers to fresh plants. Furthermore, an insect which feeds for a long period on a sugar-beet plant infected with the curly-top disease remains infective for a longer time than an insect which has fed on a diseased plant for a short period only. These facts suggest that the insect accumulates somewhere in the body a given quantity of virus which gradually decreases unless access to a fresh source of infection is obtained. On the other hand, the fact that some insects, once infective, can retain infective power for long periods, if not for the rest of their lives, without again feeding on a source of virus, suggests that in these cases there may be some increase of virus within the body. It is probably necessary to consider each case separately, and we cannot yet say definitely whether or not a plant virus multiplies within the body of the insect.

Evidence on the nature of viruses.

This problem of multiplication of virus inside the insect is really rather important and raises questions of more than academic interest. If we take the view that a virus is some kind of very minute organism, then it need not be very surprising that it can multiply inside the insect vector. If, on the other hand, we regard a virus as an unusual kind of protein, a much more likely suggestion, as we have already seen, then it is unlikely that there is multiplication of such viruses in the insect. If a plant virus is derived, as has been suggested, by some kind of conversion process of the already existing plant protein, it is also difficult to conceive of this process

taking place within the insect. It is perhaps slightly more understandable in the case of animal viruses and their insect vectors.

Why an insect as intermediary?

We still have no answer to the question why it is that some plant viruses can only be transferred by means of a particular insect and not by the simple mechanical transfer of virus sap to the healthy susceptible plant. Does this mean that the virus is altered in some way within the body of the insect, and that this alteration allows the virus to multiply within the plant? We can at least speculate on this question, and one explanation may lie in the possibility that such viruses instantaneously lose their infective power if exposed to the air in extracted sap during the process of mechanical inoculation. When transferred, however, by the sucking insect the virus would be protected from the effects of the external environment during its passage from plant to plant. This suggestion does not explain the specificity of insect vectors, or in other words, why it is only one particular insect which can transmit a particular virus since all insects of this type feed in the same way. To make this explanation plausible we must also suppose that there are other conditions inside the insect which need to be fulfilled.

In some cases it has been suggested that a particular insect can transmit a plant virus because it injects it directly into the most suitable area of plant tissue for multiplication to take place. A case in point is the virus

of the sugar-beet curly-top disease; this virus can only multiply in the phloem area of the plant, and the insect which transmits it is able to do so because it inserts its stylets directly into the phloem. This virus can also be occasionally transmitted mechanically if by a lucky chance it is conveyed straight into the phloem. Apparently it is destroyed if it comes into contact with the other tissues of the host plant.

Another possibility is that the virus requires the presence of the insect's saliva, or of some substance in the saliva, to allow the virus to start its multiplication within the plant.

What happens to the virus when it is swallowed by an insect-vector? In insects of the sap-sucking type such as aphides and leaf-hoppers it passes with the sap into the alimentary canal and cannot be regurgitated because of the oesophageal valve which prevents the outward passage of undigested food. We must therefore assume that before the virus can be ejected it has to pass through the wall of the alimentary canal into the blood and thence to the salivary glands, whence it is delivered with the saliva into the plant in the process of feeding. Some information on this movement of the virus within the insect's body is afforded by experiments with a species of leaf-hopper which transmits a virus affecting maize. The interesting fact has been demonstrated that there exist two kinds, or races, of this leaf-hopper, one of which is able to transmit the virus while the other race is unable to do so. Yet there is no visible difference, externally, in the appearance of the two kinds of insect

and both are undoubtedly the same species. Those insects which are capable of transmitting the maize virus are known as the "active" race, and those which are incapable of transmission as the "inactive" race. It has been shown by appropriate tests that the virus is present in the blood of "active" insects after they have fed upon a diseased plant but never in the blood of "inactive" insects in similar circumstances, though virus can be demonstrated in the rectum. Furthermore, if the wall of the alimentary canal in "inactive" insects be pierced with a fine needle, then insects treated in this manner do, for a time at any rate, behave as "active" vectors.

An interesting parallel to this behaviour has been demonstrated in some studies of a virus which causes a kind of "sleepy sickness" in horses. This virus, technically known as equine encephalomyelitis virus, is transmitted by certain species of mosquitoes. In this case instead of having two variants of the insect and one virus, we have two variants or "strains" of the virus and one insect. As the reader will learn from Chapter VI, viruses tend to vary or "mutate" in some degree, so that a given virus may appear in several slightly different but closely related forms. Equine encephalomyelitis virus occurs as the "eastern" and the "western" strains. The mosquito, *Aëdes aegypti*, which transmits the western strain is unable to transmit the eastern type. If, however, a puncture is made through the wall of the alimentary canal of the mosquito just as was done with the plant leaf-hopper, then the mosquito can transmit both strains of the virus. It appears therefore from these

two experiments that it is necessary for the virus to be able to pass through the wall of the alimentary canal before the insect can become a vector. It is not at all clear, however, why the wall of the alimentary canal in one type of insect should not allow passage of the virus, while in the other type it apparently does so. Nor is it clear, in the case of the mosquito, why one strain of virus can pass through the intestinal mucosa while the other virus strain cannot. These are points which still require investigation.

Several viruses in one disease.

The obligate relationship between a plant virus and a particular insect has led to some unexpected and interesting experimental results. Thus, an insect can be used to separate one plant virus out of a complex of several viruses, and it has also been used to demonstrate the existence of more than one virus in a disease, a fact which previously had not been suspected. There are two common viruses which frequently occur together in the potato plant, known as *X* and *Y*; if we feed a particular species of green-fly upon a plant affected with these two viruses and then transfer the insect to a healthy plant, the latter develops a disease characteristic of one virus only, i.e. virus *Y*. Yet since both viruses are uniformly distributed throughout the diseased plant on which the green-fly first fed, it is clear that the insect must imbibe both viruses with the sap, and the separation of the two must take place within its body. It is difficult to understand the mechanism of this separation, for in this case

both viruses are easily transmissible by sap inoculation but only one, the *Y*-virus, is insect-carried. Yet the virus which is not insect-borne is much more stable and more resistant to external factors than the one which is so transmitted.

At the moment, therefore, we can only suggest that conditions inside the body of the insect are antagonistic to the second virus, but we cannot say what these conditions are. It may be that the digestive enzymes in the body of the insect rapidly destroy the virus—it is known, for example, that the proteolytic enzymes, pepsin and trypsin, do destroy its infectivity.

It seems clear, however, that inability on the part of an insect to transmit a virus does not necessarily imply the immediate destruction of that virus within the insect's body. This has been shown by some recent interesting work on the curly-top disease of sugar beet. The specific insect which transmits the virus causing this disease is a leaf-hopper, but it has been found that an aphis, or green-fly, is also capable not only of picking up the virus after feeding on a diseased plant but of retaining it in an infective state for a considerable period. Nevertheless, the aphis is not able to infect a healthy sugar-beet plant with the virus. Again, it has been shown that several species of mosquitoes are capable of picking up the virus of yellow fever and in some cases of retaining it for about 2 weeks inside the body. But they are unable to transmit the virus by biting.

Virus inheritance.

Another interesting point in the relationship between insects and viruses is the question whether an infective parent insect can transmit the virus to its offspring so that they also are infective without ever having fed on a source of virus. In the world of plant viruses such inherited infection was unknown until quite recently, when a Japanese worker claimed that he had demonstrated this phenomenon. The insect in question is a type of leaf-hopper which transmits a virus affecting the rice plant. According to this worker some of the progeny of a pair of leaf-hoppers inherit the virus provided the female parent is the infective one of the pair. If the male is infective and not the female, then all the progeny are free of the virus. It is also stated that occasionally the virus is transmitted to the third generation when the parents had been reared on a virus-immune plant, which of course prevented the insects from re-infecting themselves. If this is true it offers, incidentally, some evidence of multiplication of the virus in the insect vector.

There seems to be no similar case of virus inheritance among the insects which transmit the animal viruses, though the process can apparently be reversed experimentally and the adult insect made to receive the virus from the larva, a phenomenon common enough in the vectors of plant viruses. In these experiments half-grown larvae of the mosquito, *Aëdes aegypti*, were placed in a mixture of blood serum from a monkey, infected

with yellow-fever virus, and an equal quantity of normal saline. The larvae turned into pupae in about 48 hours and the pupae were transferred to tubes of clean water. Adults began to emerge on the fourth day after contact with the virus. The first twenty-eight males were ground up and injected subcutaneously into a healthy monkey on the day of emergence. Four days later the monkey developed fever and had the virus in its blood. Hereditary transmission in arthropod vectors does occur in the Rickettsia diseases, though it is doubtful if these disorders should be included in the virus category. Thus the Rickettsia which causes Rocky Mountain spotted fever in the United States is passed from parent to offspring in the dog tick *Dermacentor andersoni* which transmits the disease.

Difference in virus-transmitting power during an insect's lifetime.

Among the many problems in the relationship between virus and vector which await solution is the difference in the virus-transmitting power between young and adult forms of the same insect. We have already seen that the nymphal form of the leaf-hopper is unable to transmit the virus of aster yellows, and that was shown to be due to some factor in behaviour of the virus itself. Another case in point concerns a plant virus, causing "tomato spotted wilt", which is transmitted by a minute insect known as a thrips. It has been shown that, although the adult insect does transmit this virus, it can only do so if it has imbibed the virus while in the larval state. In other words, an adult uninfective thrips

cannot become a vector by feeding on a source of virus.

It is very difficult to find an explanation for this state of affairs. It cannot be that conditions inside the body of the adult thrips are unsuitable for the virus, because it is able to persist there comfortably. Although the gullet of the adult seems slightly narrower than that of the larva, we can hardly suppose that the virus cannot move up the gullet if it can move down. We can suggest, perhaps, that the adult insect feeds in a slightly different manner from the larva, so, while able to infect the plant, cannot pick up the virus afresh. Alternatively, it may feed only on parts of plants where there is little virus: but at present these are only speculations.

Another type of differential transmission between adult and larva is to be found in the Rickettsia disease called Tsutsugamushi. In this example the vector is not an insect but a mite, *Trombicula akamushi*, and here again the larva is the transmitter of the disease, but the reasons for this are quite different. The larval stage of this mite is the only stage which feeds on an animal (the adults and nymphs are vegetable feeders). The larval mites feed on almost any kind of animal, men, rodents, shrews, dogs, cats, goats and buffaloes, and they feed only once. They do not therefore themselves infect with the disease, but the Rickettsiae are retained by the vegetable-feeding nymphs and adults and passed on to the next generation, the larval stages of which become the vectors of the disease. There seems to be an even closer association of the Rickettsiae with insects and

other arthropods than is to be found with the true viruses, and the suggestion has been made that, as a class, Rickettsiae were originally insect parasites which have come to mammals and, eventually, to man through the mediation of blood-sucking insect vectors.

From the facts briefly stated in this chapter we learn that the relationship between virus and insect vector is more than a mechanical contamination, but we cannot say for certain that the virus undergoes a definite change inside the insect. It is clear, however, that some obligate connection exists between the two, and in the case of some of the animal viruses there is evidence of multiplication of virus within the insect. Much work yet remains to be done upon this interesting aspect of the subject, especially upon the questions of the specificity of a particular insect for a particular virus, and why it is that some insects which may retain an infective virus inside their bodies for long periods are yet unable to transmit it to susceptible hosts.

CHAPTER VI

THE VIRUS AND THE LIVING CELL

Viruses can only grow in the living cell.

In any study upon virus diseases, there is one outstanding fact which must strike every investigator, that is, the nature of the relationship which exists between the viruses and the cells they infect. This relationship is so fundamental that it has given rise to the theory that some at least of the viruses are themselves the inanimate products of the disordered cells. Closely linked with these cell affinities of viruses is one of their outstanding characteristics, the fact that they cannot be cultivated in an artificial cell-free medium, such as broth or agar or any of the preparations used in the cultivating of bacteria, etc. This property differentiates them sharply from most of the known organisms.

This dependence on the living cell is well shown by the following experiment: Vaccinia virus has been proved to multiply in a medium consisting of Tyrode's solution and blood serum to which a fragment of fowl kidney has been added. If, however, the fragment of kidney is first subjected to freezing and thawing, a process which kills the cells, and then added to the medium, no multiplication of virus takes place. It is clear therefore that the cells must be living for the virus to multiply.

Since, however, the cell and not the whole organism

is the true host of the virus, it does not matter if the cells themselves are detached; the essential condition is that the cells must not be *dead* cells. It is therefore possible to "grow" viruses in small pieces of living tissue which are themselves growing in sterile nutrient medium. Such tissue-culture methods have been much used in the study of viruses and, as we shall see later, valuable information has been gained thereby.

With some viruses the connection with the cells of the host is so close that it becomes a kind of partnership in which the cells lose their allegiance, so to speak, to the main organism of which they are an essential part. In such a case the virus is actually protected by the cell and the two work actively together to produce a disease. We can illustrate this phenomenon by reference to the papilloma disease of rabbits which under certain circumstances gives rise to tumours. It has been shown that, when conditions are favourable to both cell and virus, vigorous growths arise, but such conditions do not always hold. Sometimes conditions become bad for the cells of the papilloma while remaining good for the virus which is associated intimately with them. In such cases the tumours gradually disappear in spite of the sustained virulence of the virus. It is believed that this retrogression of the tumour is due to a generalized resistance elicited by the abnormally developing cells. This generalized resistance is quite distinct from that directed against the virus itself in the production of what are known as antibodies (see Chapter VIII). It has also been observed that tumours may grow vigorously and yet be

nourished by blood which is full of antibodies and is highly destructive of the virus. Evidently the living papilloma cells protect the virus from the efforts of the main organism to destroy it. This is an excellent illustration of one of the subtle ways in which the virus acts as the true enemy of living things. Later in this chapter, when dealing with methods of tissue culture, we shall see other cases of the protection of viruses by cells.

Inclusions inside the cell.

One of the characteristics of many virus diseases of both animals and plants is the appearance in the affected cells of curious abnormal bodies which are known collectively as "inclusion bodies" or "virus bodies", and, in the case of plants, as "*X*-bodies". These bodies have been the subject of controversy among virus workers for many years, and various theories as to their nature have been suggested. They are not associated with every virus disease (they are not found in the chicken tumours for example), and they seem to be restricted to particular types of cell. Since it is not at all certain that the two kinds of cell inclusions in virus-diseased animals and plants are in any way analogous, they will be dealt with separately. In any case it is probable that one theory is not sufficient to cover all the different types of inclusion bodies. The two most obvious alternatives are first that they represent products of cellular reaction to infection or that they are the result of cell degeneration, and secondly, that they may be aggregations of the virus itself. It is probable that both these explanations are

correct. For our purpose, so far as the animal viruses are concerned, it will suffice if we describe one of these inclusion bodies, that associated with a virus disease of mice known as "infectious ectromelia". This type of cell inclusion, together with that of fowl-pox, is more suggestive of an aggregation of the actual virus than are many of the other cell inclusions.

Infectious ectromelia was described for the first time in 1930, when it appeared among some young mice at the National Institute for Medical Research. The disease first attacks the feet of the mouse, which become swollen; if the infection is restricted to the feet, the animal may recover with the loss of a toe or a foot, but if infection becomes generalized the disease is invariably fatal. The following account of the inclusion bodies is taken from the work of Marchal, who first described the disease. In sections made about the third day after infection the epithelial cells over considerable areas show numerous inclusion bodies. These vary in size from the limits of visibility to 10–12μ in length ($1\mu = 1000$th millimetre). They are usually roughly spherical when small, but the larger ones may be egg-shaped. With few exceptions they take the stain perfectly evenly and show no structure. They may occur in any of the epithelial cells of the derma, and usually more than one is seen in each cell.

In some of the virus diseases of plants there occur two well-defined types of intracellular inclusions, the one known as *X*-bodies and the other as "striate material". These two types of inclusions are particularly well shown by the tobacco-mosaic disease, and so we will use them

as our examples. The *X*-body is not unlike the cell protoplasm in appearance and is granular or finely reticulate in structure. It is well defined and in shape is rounded or elongated and is frequently in close association with the nucleus. Superficially it resembles an amoeba or similar protozoon organism, and as such it was described some years ago. There seems, however, little doubt that these *X*-bodies are really a reaction on the part of the cell to the presence of the virus. The formation of an *X*-body has, indeed, actually been observed in the living hair cells of an infected plant and a cinematograph film made of the process, which is briefly as follows. Minute particles appear in the streaming cytoplasm of the cell and are carried passively about. The particles join together, or aggregate, and so gradually fuse into a granular mass which may or may not become rounded; frequently spaces (in technical language, vacuoles) develop in the mass. The fully-formed *X*-body persists in the cell for some time but ultimately breaks down, giving a number of protein crystals which in their turn disappear after a month or two. At no time does the body show any movement of its own but is merely carried round in the cytoplasmic stream. As is characteristic of this type of cell inclusion, it is restricted in occurrence to certain kinds of tissue and is found mostly in the hair cells and epidermis.

The second type of cell inclusion associated with plant-virus diseases is of quite a different character and consists of deposits of crystalline material. If dilute acid is added to living cells containing these crystalline plates,

the latter become transformed into the needle-like para-crystals which we have discussed in the description of Stanley's isolation of the virus protein (p. 28). It appears therefore that this striate material indicates a high concentration of virus and is really an intracellular crystallization of the virus protein. In this case, therefore, it may perhaps be suggested with greater accuracy that the cell inclusion does represent the actual virus itself.

Stimulation of cell growth.

We have mentioned earlier in this chapter a disease of rabbits in which the virus causes the cells to multiply abnormally and produce growths. This type of reaction on the part of the cells occurs in one or two other virus diseases, particularly those affecting fowls. It was in 1911 that Peyton Rous discovered in a Plymouth Rock fowl a tumour which could be transmitted to other fowls. At first the transmissions were made by grafting tumour cells on to healthy fowls, and it is very interesting to record that in the beginning the tumour could only be transferred to blood relations of the fowl in which the first tumour was discovered. Later Rous showed that a filtrate which contained no cells was capable of infecting a healthy susceptible chicken when inoculated into the breast muscles and of giving rise to a new tumour. This differs from the first experiment in which living cells were transplanted because it necessitates the production of an entirely new tumour. As the experiments proceeded, the tumour lost its specificity for the blood rela-

tions of the original fowl and became infective for any variety of domestic fowl. In fact it behaved like any other virus. There are now more than a dozen fowl tumours which are caused by viruses, each virus being specific to fowls and its particular type of cell and tumour.

Stimulation of cell growth is also characteristic of certain plant-virus diseases, although nothing quite similar to tumours has yet been observed. There is a strain of the tobacco-mosaic virus which induces the leaves of infected tomato plants to give rise to small leaf-like growths on the undersides. These outgrowths or "enations" only arise on malformed leaves and under conditions of vigorous growth; they first appear as small thickened spots and their subsequent development varies. They may remain as shallow frills or small cup-like outgrowths, or they may grow into well-defined leafy structures. It is interesting to find that these outgrowths very closely resemble certain genetical abnormalities in the tobacco plant.

Growths which more nearly resemble the virus-induced tumours of fowls are produced by a virus disease of the sugar cane known as "Fiji disease". The cells which form the tissues of the larger veins on the undersides of the leaves grow excessively and produce elongated growths or galls.

Destruction of the cell.

We have seen that one effect of a virus on the cells may be to stimulate them to abnormal activity and so

to give rise to growths and tumours. Another and more common effect of viruses is the actual destruction of the cell; this is shown in the cases of many animal viruses which have an affinity for the actively growing layers of mucous membrane and skin, such viruses as those of herpes and foot-and-mouth disease, for example. In these diseases serious damage is done to the cells and scars of dead cell material are formed; in herpes, affected cells of the epidermis are swollen and show a characteristic degeneration.

The effects of certain animal viruses on cells can be examined in great detail by means of the tissue culture of viruses, a technique which we shall discuss a little later. Here it may suffice to say that the virus of louping-ill, a disease of sheep, has been cultured on the developing egg, and by this means the destruction of the cells observed. When the virus is inoculated into the egg, a lesion develops. This consists, first of a spindle-shaped thickening which is due to excess growth of cells in the outer layer. Sometimes a little ragged hole may be observed in the surface, and Burnet considers this may represent the actual cell entered and completely destroyed by the virus particles. The *necrosis*, or death of the cells, then gradually spreads outwards, and after 20 hours appears to involve about a quarter of the cells in the lesion. The affected cells are swollen and their contents are changed, the protoplasm having become granular in appearance while the cell nucleus shows various degenerative changes.

Many of the plant viruses possess this cell-destructive

or necrotic action, and in some cases it depends on the species of plant affected whether or not a given virus will exert this ultimate destructive influence on the cells. This is well illustrated in the classical tobacco-mosaic virus. When the virus is affecting the tobacco plant, its action is upon the chlorophyll with the resulting symptoms of "mosaic" mottling of light and dark green. It is only rarely under extreme conditions of high temperature that the cells are killed. If, however, the same virus is inoculated to *Nicotiana glutinosa*, a plant closely allied to the tobacco, the effect on the cells is purely destructive and numbers of isolated necrotic lesions develop on the inoculated leaves.

It is interesting to find that this cell destruction involves ultimately the destruction of the virus itself while preserving the health of the plant. A few hours after the virus has entered the leaf a band of necrotic material begins to form within the cell wall; as this necrotic barrier extends, the cells within this barrier dry up and die. The result of this is the isolation of the virus behind a ring of dead cells, and since a virus cannot move out of dead cells, it remains imprisoned in these "local lesions". In consequence there is no general spread of the virus through the plant, which remains normal and healthy.

While other viruses besides that of tobacco mosaic may produce mottling in one type of plant and death of the cells in another, there is one plant virus whose action on cells is mainly lethal. This is known as tobacco-necrosis virus, and when it is introduced into the leaves

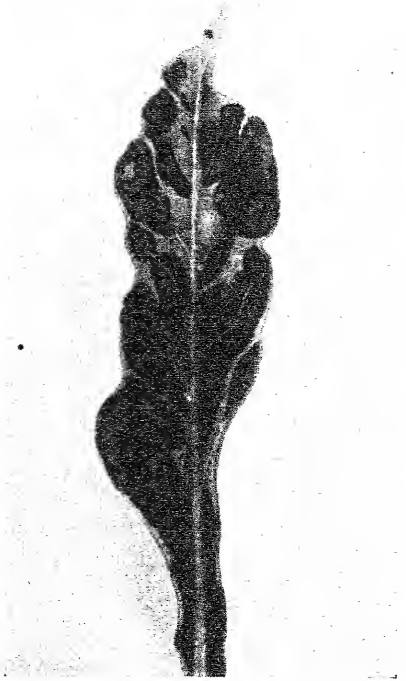


Fig. 10. A leaf of tobacco from a plant infected with tobacco mosaic: note the distortion and mottling of the leaf.

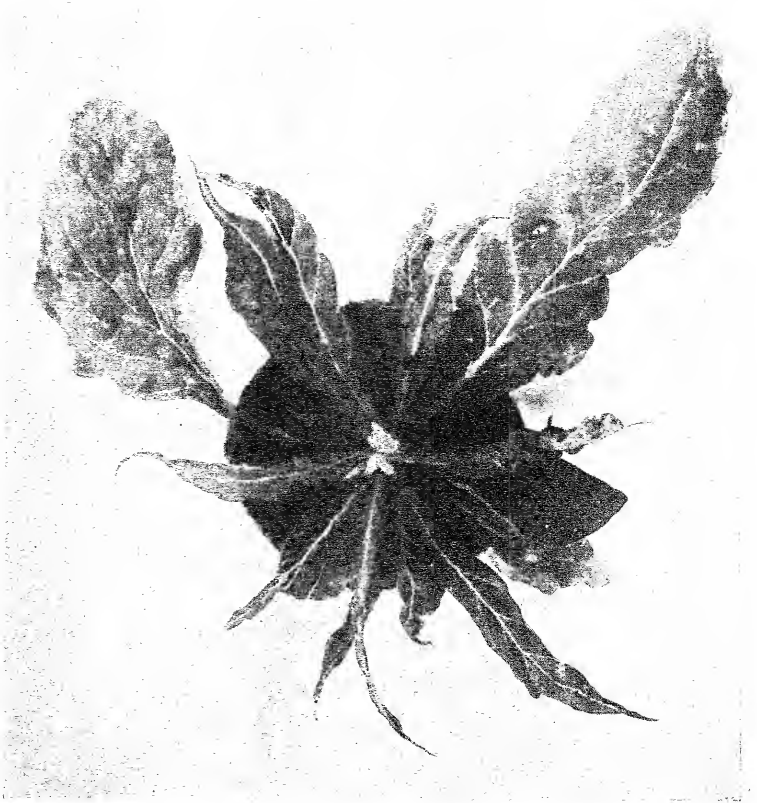


Fig. 11. Tobacco plant infected with a distorting strain of tobacco-mosaic virus: note the extreme malformation of the leaves.

or stem of a plant it produces a lesion indicating the death of the cells at that point. When this virus moves through the tissue, its path of movement is plainly etched out by the trail of dead cells. There is one type of cell, however, in which this virus apparently produces no ill-effect. That is the cells of the root, where, incidentally, it was first found.

Growth of abnormal tissue.

We have seen, then, two types of direct action of viruses on the cells, stimulation to abnormal growth and destruction. In some plant-virus diseases there is a third type, inhibition of formation of a particular kind of cell and its replacement by different and abnormal tissue. In affected plants there is usually a concentration of virus near the growing points, i.e. where new tissues are being differentiated, and it is in these areas that the first effects of the virus can be observed. In a virus disease of tobacco known as "rosette disease" one of the symptoms shown by affected plants is a lengthwise splitting of the stem, midribs and petioles. If an examination is made of the young growing points after infection but before the appearance of the symptoms, it will be observed that a change has taken place in the vascular bundles. At intervals the normal vessels are missing and their place is taken by quite a different kind of tissue. As the plant grows stresses are set up in the stem with the consequence that the stem splits under the strain.

There are other ways also in which viruses affect the cells of the host, some of which are imperfectly under-

stood. Thus, Findlay points out that in some cases there is a reduction of the host's resistance to bacterial infection, and it is interesting to find that this loss of resistance in some cases is against specific bacteria while in others it is general.

In this intimate relationship between virus and cell, not only does the virus affect the cell but sometimes the cell affects the virus. This is especially true when viruses get introduced into tissues which they do not normally enter, as for example during the tissue culture of viruses. These effects may be of various kinds; the virus may be destroyed or inactivated; it may be reduced in virulence and become a mild form: or it may be changed in other ways. In the curly-top disease of sugar beet, the virus is normally confined to the phloem region of the tissues, and if it is introduced experimentally into the parenchyma, it is destroyed. It would seem as if the other tissue cells are actually toxic to the virus. Since the question of the effect on viruses of different hosts and tissues is fully discussed later in this chapter under the heading of *variations in viruses*, we shall not deal further with the subject here but will proceed to a description of the methods of growing viruses in tissue cultures.

Growing viruses in tissues.

In cultivating viruses in tissues which are growing in a nourishing medium, one of the first difficulties encountered is the absolute necessity of keeping the tissues, and everything concerned with them, sterile, that is, free from all bacteria or other organisms. To overcome this

it is necessary for the medium and the flasks and all apparatus used to be sterilized by heat. Some of the media cannot be heated to high temperatures. In such cases the media can be sterilized by filtration which removes all micro-organisms.

In using animal tissues for cultivating viruses the investigator must be on his guard against the possibility of introducing another virus into his cultures in the actual tissues he is using. This is quite likely to happen when serum or tissues from half-grown or adult animals are used. To overcome this difficulty Li and Rivers used minced chick embryo suspended in a nutrient medium known as Tyrode's solution and contained in a special type of flask. In order to give the reader a slight idea of how the process is carried out, we can quote from the description given by Li and Rivers of their technique of cultivating the virus of vaccinia, which is the virus produced from the smallpox virus when the latter is inoculated into the calf. To obtain the necessary tissue, hen's eggs were incubated for 9 to 12 days and then opened under aseptic conditions by a special method. The Tyrode's solution was adjusted so as to be about neutral, that is, neither acid nor alkaline, and it was made sterile not by heat, but by filtering out all the micro-organisms. To contain the cultures some specially designed flasks were made, known as "collar flasks", and consisting of large vessels with perfectly flat bottoms in order to provide a wide area over which a thin layer of culture may be evenly distributed. The upper part of the flask is dilated into another smaller flask with a deep rim or

collar. The reason for this is to reduce the amount of water of condensation, while any water that does touch the cotton-wool plug is caught in the collar and so does not contaminate the culture. A small opening, leading off the collar and plugged lightly with cotton-wool, serves for ventilation; the main opening of the flask is plugged with cotton-wool and covered with tinfoil. About 1 gram of the sterile chick embryo tissue is placed in the sterile Tyrode's solution in the flask. Next, a very small quantity of virus emulsion which must also be sterile, about 0·1–0·2 c.c., is added to the flask. The whole is then incubated at 37° C. for 7 days.

That the virus does actually multiply under these conditions can be demonstrated by taking small quantities of the culture at intervals, diluting them very highly, and then testing the various dilutions on rabbits. This is done by shaving a patch of skin on the animal and inoculating each diluted sample; the presence of the virus is indicated by the development of a lesion on the skin. Multiplication of the virus is shown by the high dilution to which the cultured agent can be taken and still produce lesions on the rabbit's skin.

Tissue of rabbit embryo can be substituted for chick embryo tissue in the cultivation of vaccinia virus, and it has been noticed that the virus greatly damages the rabbit cells in which it is multiplying.

Many other animal viruses can be cultivated in a similar manner, and for the majority chick embryo tissue has proved the most useful. The virus of yellow fever can be grown successfully in chick embryo tissue in

much the same way, and the interesting discovery was made that propagation in this manner changed the nature of the virus. We shall discuss this change and its important practical applications later in this chapter.

Certain types of animal tissue are more suitable and convenient than others for growing viruses, and in 1931 two American workers, Woodruff and Goodpasture, discovered that the virus of fowl-pox could be grown successfully on a particular membrane of the developing chick embryo in the egg. Since that time many workers have reported successful propagation of other viruses by the same method, among them is that of yellow fever, and sixty consecutive transfers from egg to egg have been reported. The virus can be inoculated directly into the embryo in the egg by means of a fine needle and a special type of syringe.

An addition to this technique has recently been made by two English workers, Himmelweit and Smiles, who have made possible direct observation of the living membrane and its reactions with the virus. By this technique the infected membrane is brought into contact with the under-surface of a cover-slip closing the opening in the shell of the egg. The normal relations of the membrane to the embryo are not disturbed, and direct observation can be carried out for long periods by the aid of appropriate illumination. The "inclusion bodies" mentioned earlier in this chapter can be easily observed by this method.

Growth of viruses in single living cells.

Three American workers, Rous, McMaster and Hudack, have recently experimented on the growth of viruses in single unattached living cells. They considered that more might be learned of the relationship between cell and virus, if living individual cells were used free from the complications of a mass of organized tissue. The viruses they used were of two types, one was vaccinia virus which causes death of the cells, and the other was a rabbit virus, known as Shope's fibroma virus, which produces tumours and therefore stimulates cells to multiply.

The individual cells were isolated in the following manner. When a piece of tissue grows in some such medium as blood plasma or serum, there first forms a kind of halo of new cells round the original tissue. Now if this growth is treated with an enzyme such as trypsin, many of the proliferating cells "let go hands", so to speak, and wander off on their own. Such cells can be washed and plated in plasma, there to grow anew. In these experiments, however, the cells were exposed instead to virus-containing fluids. After the exposure of the individual cells to the virus, they were treated in various ways. First of all it was ascertained that the cells, after being in contact with the virus, always gave rise to lesions when inoculated into the experimental animals, even if the cells had been well washed before inoculation. Next the cells were killed and the experiment was repeated; again lesions were produced. Then

the first experiment was repeated, only this time the living cells, after contact with the virus, were washed with immune serum which is toxic to and kills the virus; again lesions were produced in the experimental animal. Finally, the cells were killed, exposed to the virus and washed with the immune serum; this time no lesions were produced. The experiments seem to show that the viruses become fixed on both living and dead cells and are carried through the washings with them. When the living cells are exposed to immune serum such virus as they carry is not in the least affected, whereas that associated with killed cells undergoes neutralization. Rous and his colleagues say that one is tempted to suppose that the viruses used in their experiments, after becoming attached in some way to living cells, are taken into the latter and owe their persistence in an active state to the protection afforded them inside the cell. However, the data do not justify this assumption; they prove only that the protection of the viruses is in some way *dependent upon cell life*. The maintenance of a special state of affairs at or near the cell surface might suffice for protection.

Some interesting experiments have recently been described by William Trager, in which apparently successful attempts were made to cultivate the virus of equine encephalomyelitis in the tissues of the mosquito which transmits it. These experiments are interesting from two points of view: first, because they offer evidence of multiplication of a virus in its insect vector (see p. 82), and secondly, because here we have multi-

plication of a virus without the production of any disease or apparent ill effects on the cells. In order to carry out any tissue-culture work it is of course essential that the tissues used are free from any bacterial contamination, so that the first step was to obtain the insects in a sterile condition.

The eggs of insects and the seeds of plants can be sterilized on the outside without killing them, and this is usually accomplished by dipping them in a weak solution of corrosive sublimate or in alcohol.

In the present case, "boats" were made by heating the thin glass cover-slips, used for covering microscope slides, so that the edges curled downwards. They were then sterilized with dry heat and placed in small sterile dishes containing a 5% solution of Castile soap; about twenty eggs were dropped into the "boat" and left in the soap solution for 5–7 minutes. Each boat was then lifted out with a pair of sterile forceps and put into another sterile dish containing 80% alcohol, where it remained for a quarter of an hour. Finally, each boat containing the eggs was dropped into a tube of sterile culture medium.

Trager found that when the ordinary culture medium was sterilized by heating under pressure in the autoclave, the larvae were unable to grow. Apparently, destruction of the micro-organisms by heating destroyed also one of the growth-promoting substances. This difficulty was overcome by using a special sterile medium consisting of liver extract and killed yeast; the liver extract supplied the growth-promoting substance and the yeast

supplied the larva with protein and carbohydrate. If either the liver or the yeast were omitted, the larvae failed to grow.

After the mosquitoes and larvae had been successfully raised under these aseptic conditions, portions of them were transplanted in "hanging-drop" cultures. This method is the simplest of the tissue-culture techniques and consists of placing a fragment of tissue in a drop of the culture medium on a cover-slip and inverting it over a microscope slide which has a hollow in the centre. The whole is then sealed off with vaseline or paraffin wax.

The virus grew better in some types of mosquito tissue than in others, and the best types of tissue were found to be the ovaries of newly emerged adults and the mid-gut, or alimentary canal, of full-grown larvae.

The best results were obtained with a series of cultures starting in tissue from the thorax of the larva, then transferred after 7 days to tissue from the head of the pupa, after a further 7 days to larval thoracic tissue, and 7 days later back to pupal head tissue where it was cultivated for a further 7 days. At the end of these 28 days in tissue culture the concentration of virus was 100,000 times greater than in the original suspension. Although this is a very slow multiplication compared to that obtained in chick-tissue cultures, it is greater than the increase which takes place normally in the living mosquito.

After cultivation in the insect, the virus was tested in guinea-pigs and found to be unchanged.

Variations in viruses.

It is now an accepted fact that many of the viruses affecting animals and plants do give rise to variants which, while retaining some of the properties of the type virus, yet possess marked characteristics of their own. The classical examples of variations in viruses are the production of vaccinia virus when variola (smallpox virus) is inoculated into the calf and of the "fixed" virus of rabies from the ordinary or street virus by continued brain to brain passage in the rabbit.

It is not yet possible to explain the nature of these changes in viruses. They may be mutations, though it is difficult to conceive of there being numbers of genes in some viruses, or they may be side-products of a chemical reaction, since the strains of plant viruses seem to consist of related proteins.

For our present purpose it must suffice that these variants do occur, and it may be of interest to see how some of them arise. In his comprehensive review of variation in animal viruses, Findlay classifies the forms of variation into four groups thus:

(1) Variation in virulence.

(2) Variation in the type of tissue lesion produced in the host.

(3) Variation in antigenic character.

(4) Variation in the type of lesion and in antigenic character.

In the first group we are considering only increase or decrease in virulence unassociated with other forms of

variation. Increase of virulence shows itself in an adaptation of the virus to new hosts, whereby, for example, influenza virus can infect mice, the Rous chicken sarcoma can be transmitted to turkeys and pheasants, and the papilloma virus of cotton-tails becomes infectious for rabbits. Decrease in virulence is exemplified in the mildness of the disease caused by vaccinia as compared with smallpox and by certain strains of yellow-fever virus.

In the second group a different type of symptom is produced by the variant virus, and in some cases the kind of tissue affected is also changed. For example, the so-called pantropic and neurotropic strains of yellow fever not only produce different kinds of lesions but also have become unadapted each for the tissues which the other strain affects. In the case of Shope's fibroma virus, a variant has appeared which produces lesions instead of the growths usually associated with the virus. Here, however, the tissue affinities are not altered.

The virus of foot-and-mouth disease affords a good example of variants in the third group where antigenic character alone is concerned. There are several strains of this virus which produce identical lesions but afford no immunity, one against the other. The various strains of influenza virus fall into the same category.

When difference in symptoms is associated with antigenic differences, as in our fourth group, then to all intents and purposes we are dealing with a new virus. Such an occurrence is probably rare, but there seems to be no good reason why it should not happen.

We have sketched briefly the kind of change which takes place in animal viruses, but we have not yet seen *how* some of these changes can be brought about.

When a virus is introduced into a new environment a change may take place. Vaccinia virus is produced when variola (smallpox) virus is passed through a monkey and then to the skin of the rabbit, or alternatively the skin of the calf. Although it seems highly probable that the smallpox virus actually gives rise to the vaccinia virus, another possibility must be borne in mind. It may be that the new environment exercises a selective rather than a formative action and thereby enables the vaccinia virus to develop at the expense of the smallpox virus. This theory of course presupposes the co-existence of the two strains.

The production of vaccinia virus is an illustration of a variant arising in the same tissue but in a different species, i.e. the skin of the calf instead of the skin of man. Variants may also arise when a virus is introduced into different tissues either of the same or another species. Rabies virus offers a good example of this type of variant, and Pasteur showed that the so-called "fixed" virus could be obtained at will by passage of the "street" virus through the brains of rabbits. Similarly, the virus of yellow fever can be made "fixed" for nervous tissues by propagation in the brains of mice.

Virus variants sometimes develop under tissue-culture conditions where the virus is growing in a different species and a different tissue. One of the most important of these variants is a modified type of yellow

fever. The virus can be made to lose its affinity for nervous tissue, and it is thought that the modification induced in cultivated strains of yellow-fever virus is determined by the nature of the tissue used in the medium. The importance of this discovery is discussed further in Chapter VIII in dealing with the control of virus diseases. Occasionally viruses can be "educated" to infect an animal; it is not possible, for example, to transmit the virus of influenza directly from man to mouse, but the virus can be induced to infect mice if it is first passaged through a ferret. Sometimes it is necessary to pass the virus through several ferrets before it becomes virulent for mice.

What are thought to be spontaneous variations occur at times; one such variant appeared quite suddenly in the fibroma disease of rabbits. Instead of giving rise to fibromata, the virus commenced to produce lesions and has remained "fixed" for lesions ever since. These apparently spontaneous variations occur quite commonly in certain plant viruses, particularly those of the tobacco-mosaic type. Diseased tobacco plants exhibiting a green mottling of the leaves sometimes develop a yellow spot amongst the green. If a subculture is carefully made from this yellow spot, by pricking through it with a sterile needle to a leaf of healthy tobacco plant held underneath, a virus is isolated which produces an entirely different disease consisting of a bright yellow mottle instead of the normal green. Provided the "yellow" virus has been isolated without any accompanying "green" virus it remains true to type

and continues to produce yellow mottling. If, however, during the isolation process some "green" virus contaminates the "yellow", the latter is eventually submerged by the more actively multiplying "green" virus.

The protein molecules of these strains of tobacco-mosaic viruses while resembling those of the type virus yet show certain differences.

Other variants in plant viruses can be produced by artificial means; an attenuated strain of tobacco mosaic develops when a plant affected with the type or parent strain is grown at a very high temperature. This attenuated virus seems to be fixed and does not revert to the normal type.

We have seen that some animal viruses are changed by passage through different species, and we can find a parallel to this phenomenon in the plant viruses. The virus of curly-top of sugar beet can be attenuated by passing it through certain species of plants such as *Chenopodium murale* or *Rumex crispus*. Such attenuated viruses can then be reactivated to their original virulence by passage through the chickweed, *Stellaria media*. A somewhat similar type of attenuation can be produced by heating the virus for 10 minutes at 76–79° C.

In experiments at Cambridge, two common potato viruses, known as *X* and *Y*, have been passed continuously through tobacco over a period of years. It is now extremely difficult to induce these strains of the two viruses to infect the potato plant although this was the original host. During long cultivation in the tobacco

plant the viruses seem to lose to a great extent their ability to infect the potato. In addition the *Y*-virus has become extremely attenuated either through the development of a variant or through some process of selection. The virus is in fact now so mild that the disease it produces can only be detected with difficulty.

New virus diseases.

We have previously stated that the appearance of new viruses and virus diseases is a possibility, and this may occur through variation which is sufficiently wide to produce a "new" virus. New virus diseases may arise by the transfer of an existing virus to a new host or conceivably by the development of parasitic habits by a non-parasitic virus, if such exists.

Findlay mentions some interesting cases of apparently new virus diseases of animals. Thus, in the United States and in the Argentine there occurs a virus disease of horses to which we have had occasion to refer already, equine encephalomyelitis. Two strains of this virus occur, an eastern and a western, which are immunologically distinct although they produce very similar symptoms. Under natural conditions these two viruses are not known to infect any other animal, and they are quite distinct from the virus diseases affecting horses in the Old World. Now horses or their ancestors, for reasons unknown, became extinct in the New World at the end of the Pleistocene period, and therefore were absent from the New World till they were reintroduced by the Spanish conquistadores. Equine encephalomyelitis must

therefore have originated in America since the Spanish conquest. Similarly, a fatal virus disease of rabbits, known as infectious myxomatosis, has also arisen in the same period, for true rabbits have never existed in a wild state in America and hutch rabbits were only introduced by the Spaniards.

In the plant world "new" viruses appear at intervals, though their appearance can more easily be explained on the assumption that they have either been introduced on some plant from another country; or else that a virus, previously infecting some weed or wild host plant, has been brought into contact with a cultivated susceptible crop and by this means comes under notice. A few years ago an entirely new virus disease suddenly appeared among the tomato plants of a Bristol grower. The virus causing this disease, now known as tomato bushy stunt, had never been described anywhere in the world before. Moreover, except for a few sporadic attacks during one or two years following its first appearance, the disease has not been recorded since. The virus itself, however, has been preserved in various research stations throughout the country and is the one which has been purified in a truly crystalline state (see Chapter II).

Since a virus is not possessed of any motive power of its own, the question arises as to how such an agent moves out of the cells it first enters and becomes distributed throughout the whole organism. The question of how viruses move through plants has been much studied, and various ingenious experiments have been designed

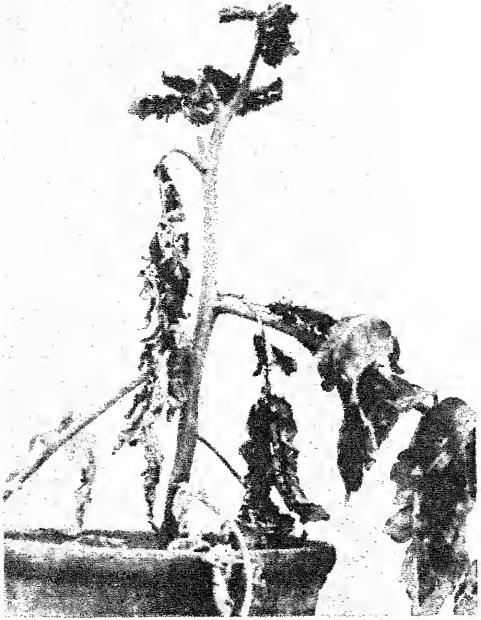


Fig. 12. Tomato spotted wilt, a serious virus disease of the tomato and many other plants.

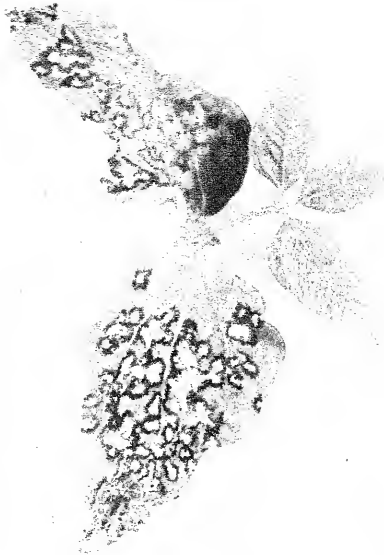


Fig. 13. The effect of rubbing the leaves of the cowpea with the virus of tomato bushy stunt: note that the virus does not move out of the rubbed leaves. This is the virus shown in crystalline form in Fig. 5.

to investigate the matter. In one such experiment the stem of the plant was ringed by applying steam or molten wax in such a way as to destroy all living cells in a ring round the stem while leaving the non-living water-conducting vessels intact. When the plant was inoculated in the portion *above* the ring of dead tissue, the disease developed only in that area; similarly, the disease appeared in the lower part of the plant only when inoculation was made below the ring. In other words, the ring of dead tissue offered an insurmountable barrier to the passage of the virus. The result of this and other experiments suggests that the virus is carried throughout the plant in the phloem and is thus closely connected with the movement of food materials about the plant. This, however, is not quite the whole story of how the virus gets distributed. When a leaf of a healthy tobacco plant is lightly rubbed with a finger moistened with tobacco-mosaic sap, the virus enters the leaf and a few days later can be recovered from any part of the plant. There must therefore be some mechanism which enables the virus to pass out of the primarily infected cells and so reach the phloem. Now connecting each cell with its neighbour are some very fine protoplasmic strands known as "plasmodesms", and there is a certain amount of evidence which suggests that the virus is carried along these "bridges" from cell to cell and so gradually reaches the phloem by means of which it is carried all over the plant.

The methods of spread of animal viruses are varied; yellow-fever virus is carried in the blood stream, other

viruses such as those of rabies, poliomyelitis and herpes travel in the nerve fibres, and there is one virus which moves in the lymphatic canals. Some viruses infect cells at their portal of entry while others travel both by the blood stream and by nerves.

CHAPTER VII

IMPORTANT VIRUS DISEASES

We have mentioned previously that almost every type of living organism is liable to attack by virus diseases. Thus man, all kinds of domestic animals, birds, fish, insects, plants and even bacteria are all known to be susceptible to these disease agents. In the Appendix on p. 174 a list is given of the more important virus diseases of animals and plants.

In man.

In this chapter a description is presented of one or more important virus diseases attacking each of the great groups of living things, and a short account of the bacteriophage is also included. It is outside the scope of this book to give detailed clinical descriptions of the virus diseases of man, and since no good purpose will be achieved thereby we will only state that these diseases can be loosely grouped together according to the tissues affected. Fine has classified them as follows:

(1) *The Dermotropic Group* (affecting skin or mucous membrane only): the viruses of warts and molluscum contagiosum.

(2) *The Neuro-dermotropic Group* (affecting and capable of infecting the nervous system): the viruses of herpes, zoster, varicella and smallpox.

(3) *The Neurotropic Group* (affecting nervous system only):

the viruses of acute anterior poliomyelitis, rabies, encephalitis lethargica (sleepy sickness).

(4) *The Respiratory Group* (affecting respiratory tract): the viruses of mumps, measles, influenza, common cold and psittacosis (parrot fever).

In the cow.

In cattle one of the most important virus affections is foot-and-mouth disease. After the onset of infection the temperature of the animal rises to about 40° C., the vesicles which are a characteristic of the disease begin to appear and the fever abates. McKinley describes the course of the disease as follows: While lesions in the mouth are commoner in cattle than in sheep, goats, and hogs, the buccal mucous membrane becomes sensitive and the animal may cease to eat. The mucous membrane of the lips and gums is dry and reddened; saliva accumulates in great quantity and falls from the mouth when it is opened. Usually on the second or third day after onset of symptoms, vesicles appear which at first contain a clear watery fluid that later becomes greyish white. After one or two days the vesicles break and a painful flat erosion remains. These erosions soon become covered with new epithelium and the animal commences to eat once more. In cattle, vesicles also develop on the muzzle at the base of the horns and in hogs upon the snout. Hoof infection usually develops at the same time or directly following the eruption on the mucous membranes of the mouth. At first the animal shows signs of lameness and the affection may be so severe that the

animal refuses to walk at all and lies on the ground. Vesicles appear on the skin of the coronary band, the heel of the foot, and the interdigital space. These vesicles usually burst before those in the mouth and become mixed with dirt and form crusts. New epithelium is gradually formed and healing with scab formation takes place. In cattle the vesicular lesions frequently appear upon the udders. These lesions may become serious and lead to painful swelling of the udder and catarrh of the milk duct.

In general the great majority of cases of foot-and-mouth disease run a favourable course. In uncomplicated cases the disease runs its course in 2–3 weeks. While some cases may be left permanently affected with chronic disturbances of the hoof, the majority recover completely.

This virus is extremely infectious and it is passed from diseased to healthy animals by contact, in droplets and in the excretions of infected animals. There is some doubt, however, as to the exact manner in which the virus is carried over distances, as it presumably must be in order to account for isolated outbreaks of the disease (see p. 73).

In the pig.

Swine influenza, or "hog-flu", is an important and interesting disease, because it has been proved that this disorder is due to the combined attack of a virus and a small bacillus. Moreover, the virus element bears a close relationship to the virus of human epidemic influenza.

Sera from a very high proportion of human adults has been found to neutralize the virus of swine influenza, and Laidlaw has made the interesting suggestion that the virus of swine influenza is really the virus of the great human pandemic of 1918, adapted to the pig and persisting in that species ever since. Shope of the Rockefeller Institute, Princeton, has shown that the virus is harboured by parasitic lungworms infesting pigs. These worms spend part of their life-cycle in the pig and part in the earthworm. A healthy pig can thus become infected with the virus by eating earthworms containing these parasitic worms in which the virus is lying latent.

In the dog.

Distemper is a well-known and important disease of young dogs, and has been recognized since the days of Jenner. The symptoms of the disease are rather variable, probably because of invasion by secondary infective agents. So widespread is this disease that it is difficult for workers on the subject to obtain healthy, susceptible animals, since the majority of young dogs collected at hazard have recovered from the disease. Such dogs are immune to further attacks and are thus useless for experimental purposes. In carrying out their classical researches on dog distemper, Dunkin and Laidlaw found it necessary to use a stock of dogs bred under strict isolation with elaborate precautions to prevent accidental infection.

The experimental disease differs slightly from the natural disease, and Laidlaw defines it as an acute in-

fectious fever characterized by an incubation period of only 4 days, a coryza (acute nasal catarrh) at the onset of the disease, an unusual temperature curve, severe gastro-intestinal disturbance, and a variable set of symptoms due to inflammation in the respiratory tract. In a proportion of cases nerve symptoms caused by an encephalitis may develop.

The disease, as it occurs under natural conditions, differs slightly from the foregoing, and the incubation period seems to be much more prolonged. The catarrhal symptoms are more pronounced and the death-rate from secondary infections such as pneumonia is much higher than in the experimental disease. Distemper in dogs seems to have much the same effect in lowering resistance to bacterial infection as influenza does in man.

Dog distemper occurs wherever there is a canine population, and epizootics develop from time to time. Almost every year there is an outbreak among the young foxhounds on their return from "walk", where they have been more or less in isolation and so have not been exposed to infection. Since the work of Dunkin and Laidlaw (see Chapter VIII) on the control of distemper, however, the importance of the disease has been much reduced.

As in the case of foot-and-mouth disease, there is some doubt as to the means of spread of the virus over distances during an epizootic. There is no evidence to incriminate ectoparasites as vectors, and the virus itself is fragile enough although it has been shown to travel short distances in the air.

In his review of the work of Pasteur, Dr Sambon gives a graphic description of that dreadful virus disease, rabies, and its effects on the dog:

"With the development of the disease the dog's disposition changes. He becomes depressed, dull, uneasy, his attitude indicating suspicion and fear; he creeps away into dark corners or under beds. Then, agitated and restless, he moves from place to place, lies down and gets up; when about to lie down, he turns round and round far more than the bed-making instinct requires; indeed he demolishes his bed and changes posture again and again. He sniffs about anxiously, scratches with his forepaws, tears curtains and carpets, gnaws wood and leather as avidly as a puppy, swallows inedible things such as hair, straw, rags and stones.

"Suddenly he is off at a slouching lope, tail down, head and ears drooping. He wanders erratically far from home, running, stumbling, stopping with neck outstretched, growling, snapping at the air, or springing as if to seize a prey, eyes red and glaring. His bark is furious but unnatural, reverting to the dismal call of the wolf; it is a jumble of bark, howl, growl and snarl, followed by four or five diminishing howls, a sinister, lugubrious sound, once heard never forgotten.

"Agitation increasing, exasperated, the impulse to bite is irresistible and he will fly savagely at any animal or man in his path. Thus often travelling many miles, he may leave a trail of death behind him. He appears to be harassed by phantoms; movements and attitudes both suggest hallucinations of sight and hearing.

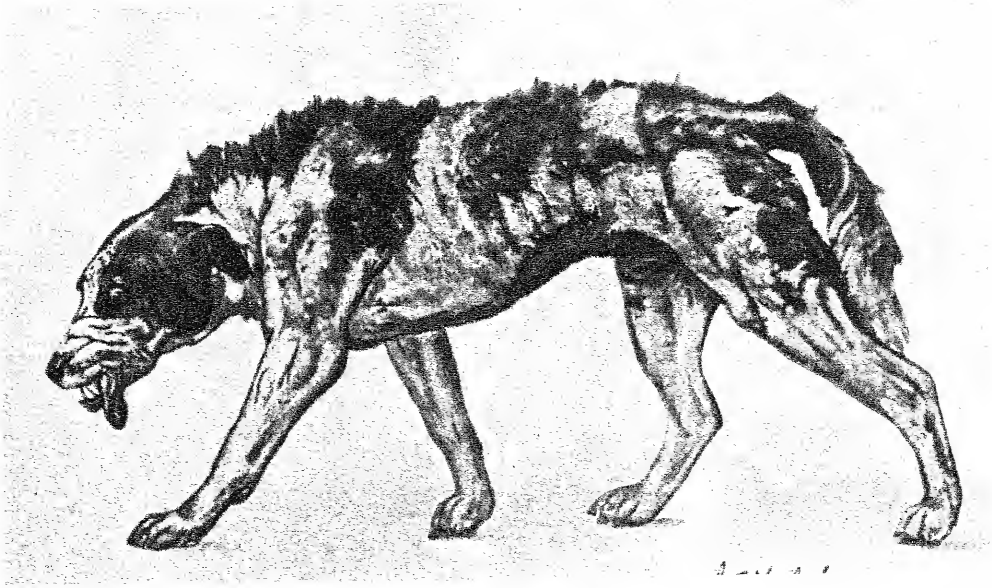


Fig. 14. Study of a rabid dog, from a painting by J. T. Nettleship.

"It is at this stage, in spite of himself, that he is likely to bite his master. His bloodshot eyes with wide pupils, squinting and glinting, have a baleful expression. The face and forehead are wrinkled, a frothy viscid saliva hangs in strings from his curled-up lips; he is gulping constantly as if trying to swallow something; his laboured breathing is marked by strange choking sounds. Between paroxysms of insane rage he falls exhausted.

"In cases of so-called 'dumb madness' there is no barking; the lower jaw drops paralysed with lolling lead-coloured tongue and dripping saliva. The animal crouches with eyes staring and fixed. After only a few days, fearfully wasted, back arched, fur bristling, covered with dirt, the rabid dog moves stiffly, swaying, staggering and falling, finally to move no more."

Very similar is the picture presented by the human sufferer, and it was from this dreadful scourge that mankind was liberated by the genius of Pasteur. Rabies is no longer present in England and need never be again unless it is reintroduced by means of dogs smuggled into this country in criminal disregard of the quarantine laws.

In the mouse.

Mice are susceptible to a variety of virus diseases, but many of these are experimental infections since the mouse is largely used as a laboratory animal. We have, however, already mentioned one naturally occurring virus disease of mice, infectious ectromelia, when discussing the intracellular inclusion bodies.

This disease was first noticed and described by Marchal as occurring naturally, mostly among young mice in the breeding stocks at the National Institute for Medical Research. The disease first shows itself as an enlargement of one foot, usually a hind-foot. The swelling of the foot is considerable and there is a transparent appearance indicating oedema (a dropsical condition of the cells) beneath the skin. This condition rapidly increases so that the foot may become hemispherical in outline. In the next stage the foot becomes moist, and serous fluid escapes on to the surface forming minute scabs. There is frequently a constriction between the healthy and diseased skin. As the disease progresses either the swelling tends to disappear and the animal recovers completely with perhaps the loss of a single toe or the foot dries up and separates from the limb at the line of constriction. Occasionally another foot or all the feet become infected, or similar lesions may appear on the tail or round the mouth. Such generalized infection is invariably fatal.

Marchal noticed that in an infected community the death-rate was extremely high even amongst mice showing no external signs of the disease. Post-mortem examination of these mice revealed the fact that the animals showed definite changes in the liver and spleen which have now been proved to be due to the virus disease. It appears, therefore, that the external symptoms on the feet only develop in a percentage of cases.

The natural method of spread is not yet completely elucidated, but it seems highly probable that it spreads

by contact. When infected mice were placed in a box together with a number of normal animals, the mortality among the latter was extremely high, though only a small percentage developed the characteristic symptoms on the feet.

Some experiments were carried out to see if the bed-bug, which is frequently present in rooms containing animals, was able to transmit the virus. These experiments, however, were completely negative.

In the parrot.

Many readers will recollect the alarm which was caused about 10 years ago in this country by the outbreak of "parrot fever", or, to give its correct name, Psittacosis. This virus affects parrots, budgerigars, Java sparrows and even canaries and is also very easily communicable to man in whom it produces a severe disease. Not much has been heard of this virus of late years but that it is still about is shown by its appearance in the parrot house at the Zoological Gardens in London last year.

The first outbreak of psittacosis in man seems to have occurred in Switzerland in 1879 and was followed by a more serious outbreak in Paris in 1892. In the first case parrots were suspected as the source of infection, and in the second outbreak all the human cases could be traced to a single consignment of parrots from Buenos Ayres, when five hundred birds were exported of which three hundred died en route. In the English outbreak of 1929 there is little doubt that the infection was brought into the country in parrots from Brazil.

The disease caused by psittacosis in man is of a severe character, resembling typhoid fever in some particulars and frequently complicated by pneumonia.

While psittacosis in man always attacks the lungs, in parrots this is rarely the case, and the most characteristic changes are found in the liver and spleen. The liver is enlarged and shows fatty degeneration, and it is frequently studded with white dots of varying sizes. The spleen also is greatly enlarged and very friable.

Infection probably takes place by the nose and mouth; the droppings of diseased birds contain the virus and are highly infectious.

The green Amazon parrot seems to be the chief but not the only source of infection, and transmission of the virus is probably by contact. Actual contact does not seem to be always necessary, since it is possible for man to become infected after merely being in the same room with an infected bird. A parrot can apparently remain infective for so long as 8 months without itself showing signs of disease.

The virus of psittacosis is interesting because it is the largest virus known and seems to undergo some kind of developmental cycle. When it is transmitted experimentally to the mouse, it can be shown that multiplication is associated with morphological changes which follow a definite sequence. Bedson states that it grows in the cytoplasm of susceptible cells in circumscribed colonies enveloped in varying amounts of homogeneous inclusion material. He concludes that psittacosis virus is a living micro-organism with an intracellular habit of life, in other words a highly specialized parasite.

In the domestic fowl.

Among other birds the domestic fowl is susceptible to attack by several virus diseases, notably fowl-pox, fowl plague and the Rous fowl sarcoma. The last-named is of unusual interest and is dealt with separately later in this chapter.

Fowl-pox is an eruptive disease which bears a superficial resemblance to human smallpox and other pox diseases of mammals. This, as can be imagined, must have caused considerable alarm in earlier times when smallpox possessed a much greater significance than it does now.

The suggestion that the two diseases were identical was disproved, partly by Spinola in 1858 who failed to transmit cow-pox (vaccinia) to birds and partly by Bollinger who was unable to transmit fowl-pox to sheep or goats. During later years the discovery of quite different types of "inclusion bodies" associated with the two diseases was further evidence that two distinct viruses were concerned, though the possibility of a relationship between vaccinia virus and fowl-pox virus must still be considered. The disease is most commonly seen in fowls and pigeons, but it also attacks turkeys, geese, ducks and guinea-fowls.

Findlay describes the course of the disease in fowls as follows: In the naturally occurring disease nodular lesions appear first on the comb, wattles, eyelids, angles of the beak and oral orifices, except in very severe cases when any portion of skin, feathered or unfeathered, may

be affected. At first the skin lesions are small, firm, greyish or pink nodules which rapidly increase in size, from about 1 mm. to about 0·5 cm. in diameter, become rough, brown or yellow in colour, and horny in consistence. If the bird survives, the nodules dry up in 2–3 weeks and later fall off as crusts without leaving a scar, except in severe cases. When the conjunctiva is affected it appears swollen, while a serous discharge pours from the conjunctival sac. Inflammation of the cornea may occur. Sometimes greyish white patches of false membrane appear on the mucous membrane of the mouth and pharynx and on the sides of the tongue constituting the so-called avian diphtheria.

Very characteristic "virus bodies" are associated with fowl-pox. They occur in the cytoplasm of the epidermal cells of the lesions and are called "Bollinger bodies" after Bollinger who gave the first description. It has been shown that a single one of these bodies when isolated and washed in saline solution produces typical fowl-pox when inoculated into the skin of a fowl.

The disease is very widespread and epidemics have been recorded from many different countries. The virus probably spreads by contact of diseased and healthy birds, though a small wound seems necessary for infection (see Chapter IV, p. 55).

Certain blood-sucking flies, such as mosquitoes and the stable-fly, are said to transmit the disease by carrying the virus mechanically on their mouthparts.

Fowl plague or "bird pest" was first reported in Italy in 1878, and since that time there have been outbreaks

reported at intervals from many countries. Of recent years, however, the disease has tended to become less prevalent. The virus attacks only birds, chiefly fowls, turkeys and geese, but many other species are susceptible, including ducks, pigeons, pheasants, guinea-fowl, blackbirds, sparrows, sparrow-hawks, owls and parrots.

According to Rice the incubation period of the disease varies from 3 to 5 days, and the symptoms are not characteristic enough to serve to distinguish the disorder from certain other bird affections.

Infected fowls isolate themselves and seek any available shade. They show no desire for food and stand or lie in a semi-comatose state with wings and tail drooping, head drawn in and eyes wholly or partly closed. Certain symptoms, such as catarrh, probably due to accumulation of mucus in the mouth, false membranes and a dropsical condition of the head and neck, have been attributed to fowl plague, but it is not yet certain whether these symptoms may not be connected with secondary infections. Rice describes certain nervous symptoms as always prominent in the disease. The bird staggers and finally is unable to stand. It exhibits involuntary movements of the head and neck, varying from slight twitchings to violent spasms.

The exact mode of infection of fowl plague is not known. The disease seems to spread by contact of diseased and healthy birds, and infection can arise from contaminated cages and equipment. There is also the possibility that wild birds might introduce the virus into a flock of fowls.

We have had occasion to refer more than once to a mosquito-borne virus disease of horses known as equine encephalomyelitis, and it may seem somewhat out of place to refer to it again here when dealing with the virus diseases of birds. Not long ago, however, two American workers, Fothergill and Dingle in south-eastern Massachusetts, noticed that large numbers of pigeons were dying off in some kind of epidemic. As soon, however, as the cold weather set in, the losses of pigeons began to cease. Just about the time of the pigeon epidemic there was also an epidemic of equine encephalomyelitis together with a number of cases of human encephalitis. Inoculation tests made to mice from one of the dead pigeons showed that the cause of the pigeon epidemic was the virus of equine encephalomyelitis which was brought to the pigeons by virus-carrying mosquitoes. During cold weather, of course, the activity of the mosquitoes would cease and with it the pigeon epidemic. This is merely another example of the unfortunate ability of viruses to attack widely different hosts, and it is to be hoped that this ability will not evolve much further. It is already possible for man to be infected with influenza from ferrets, with psittacosis from parrots and now presumably with encephalitis from pigeons.

In the rabbit.

Rabbits are liable to attack by several viruses both by experimental and natural infection. Infectious myxomatosis is a highly fatal disease of the domestic rabbit,

and it is interesting because it has some of the characters of an acute infection and also of new growths. This virus has affinities with another virus affecting rabbits, fibroma, and it is possible that one arose out of the other as a variant or mutation.

The suggestion has been made that rabbits with infectious myxomatosis might be imported into Australia in the hope of setting up an epizootic there, and in so doing reduce the numbers of rabbits.

There is a curious infection of rabbits, known as Virus III; this was brought to light by Rivers and Tillett when they were inoculating rabbits with material from chicken-pox patients. These workers carried on successive transfers through the testes and then discovered that they were transferring an agent which produced fever in the rabbits but had nothing to do with the chicken-pox patients. Andrewes and Miller also discovered the same agent when they were making a series of inoculations with apparently normal rabbit blood. The presence of the virus is never made evident until after the third or fourth passage, and Andrewes considers that the virus is carried in small quantities, possibly in the testes, by some rabbits in which it may be a residue from a natural infection. The subsequent passages of the virus through susceptible rabbits probably increase its virulence and so make its effects obvious.

In fish.

Very little serious study has been carried out upon diseases of fish which are thought to be caused by viruses.

For this reason the evidence for the existence of viruses affecting fish does not rest upon so secure a scientific foundation as is the case with the other diseases discussed in this chapter. McKinley describes three disorders of fish which are considered to be due to virus infection. No filtration studies have been carried out on these diseases, so that we have to rely largely on negative evidence for including them in the virus category. In each case all attempts to isolate any kind of organism which might be the cause of the disorders have failed. Furthermore, in one or two cases intracellular inclusions which are considered characteristic of virus infections have been described.

In epithelioma of *Barbus*, a tumour develops on the lip of the fish; there is usually only one tumour present, but there may be more. Keysselitz has studied the microscopic structure of these tumours and describes minute intranuclear bodies which he states are similar to the cellular inclusions of known virus diseases such as vaccinia, epithelioma of fowls, etc. It is by this analogy therefore that the epithelioma of *Barbus* is included here as a virus disease.

A disease of fish called "carp-pox" has been known since the Middle Ages. The external symptoms are mainly on the skin and consist of milky, glassy spots which are separate at first but later may coalesce. Hofer considers that the skin symptoms are only secondary, and that the real focus of infection lies in the kidneys which are filled with parasites often of a protozoon nature. On the other hand, inclusion bodies have

been described by Loewenthal as occurring in the skin lesions.

Lymphocystic disease of fish is characterized by the development of whitish nodules on the skin especially on the fins. Each nodule is composed of a giant lymphocystic cell covered by connective tissue and epithelial cells. After 7 or 8 months the nodules are fully developed and tend to degenerate; complete healing may occur and the lymphocystic cells are then cast off. Provided that no vital organ is affected the disease is not serious. Intracellular inclusion bodies of the usual type are present in the young lymphocystic cells.

The disease is infectious and can easily be transmitted to bass and flounders.

In insects.

We have seen already that insects have a special relationship with viruses affecting both animals and plants, in that they transmit these agents from diseased to healthy organisms without themselves suffering any apparent ill effects. We come now to consider a different relationship between insects and viruses, a condition in fact similar to others dealt with in this chapter where an actual disease is provoked.

The virus diseases of insects have been known since about 1857, but for many years they were attributed to the attacks of pathogenic organisms such as Protozoa and bacteria. It was not until about 1912, twenty years after Ivanovski's discovery of the tobacco-mosaic virus had made possible the conception of such a disease agent,

that filtration experiments showed that certain diseases of insects also belonged to the virus category. There are three general types of insect virus diseases: (1) the sacbrood disease of honey bees; (2) the so-called polyhedral diseases of Lepidoptera, and (3) two diseases of *Pieris brassicae*, the large white butterfly.

The viruses appear to infect only the immature stages, the larvae and pupae of insects.

Glaser gives an accurate account of the sacbrood disease of bees, and the following description is modified from his account. Sacbrood of bees must not be confused with "foulbrood" which is caused by a specific organism. In the early stages of the disease the larva assumes a slightly yellowish appearance and appears transparent at the lateral and posterior margins. If the body wall is ruptured, the contents of the "sac" flow out as a granular slightly milky fluid, the granular appearance being due to the presence of fat cells. The colour of the dead larva darkens and the contents consist of a granular brownish mass suspended in a watery fluid. The appearance of the remains of the larva at about this stage of disintegration best characterizes the disease sacbrood. The cuticular sac is now quite tough, permitting the removal of the larva from the cell with ease and with little danger of rupture. In the final stage of the disease the dead larva has lost all its moisture by evaporation, leaving a dry mummy-like mass known as the "scale" which can be removed or even shaken from the cell.

Sacbrood is an infectious disease, and since it has been shown that syrup containing the virus produces the

disease when fed to larvae, it must be assumed that infection would ensue when food or water used by the bees becomes contaminated with virus.

The "polyhedral diseases" are so named because the body fluids of caterpillars, which have died from the disease, contain immense numbers of polyhedral bodies of various sizes. These disorders have been studied in some detail in the silkworm (*Bombyx mori* L.), in the nun moth (*Lymantria monacha* L.), and in the gipsy moth (*Porthetria dispar* L.). The polyhedral disease of silkworms is called "grasserie" in Italy, "jaundice" in France, and "Gelbsucht" in Germany, while in America a similar disease of the gipsy moth is called "wilt".

Silkworms, before dying, are usually covered with lemon-yellow blotches and the skin assumes an opaque, shiny, yellowish appearance. Affected nun-moth larvae show few recognizable symptoms other than a loss of appetite and a tendency to migrate to the tree tops where they die in large numbers and remain hanging by their prolegs. For this reason the Germans call the disease the "Wipfelkrankheit" (tree-top disease).

Like most virus affections the polyhedral diseases are very infectious, and the virus can be passed from caterpillar to caterpillar indefinitely by inoculating them with traces of infected blood even when diluted to one in a million and by feeding them with infected material. By analogy with the crystalline plates found in the tissues of mosaic-infected tobacco plants and which seem to be concentrations of the virus protein itself, one might per-

haps ask whether the curious polyhedral bodies associated with these caterpillar diseases also represent the virus in concentrated form. Glaser has shown, however, that this is not so; he collected large quantities of polyhedra by spinning them out of the diluted body fluids of dead caterpillars on the centrifuge, and washed them carefully several times with saline. When they were injected into healthy susceptible caterpillars, they failed to produce the disease.

Polyhedral diseases are easily transmitted, the natural method being the ingestion of infected material with the food. An Italian worker, Acqua, has recently carried out some interesting experiments on the isolation of silkworms from infection. He fed the silkworms on lettuce leaves in midwinter, at a time when there were no other larvae being reared in the neighbourhood, and he kept them in cages freshly constructed of new material. Nevertheless, more than 90 % of the caterpillars reared under these conditions of isolation succumbed to polyhedral disease. On the evidence of these experiments Acqua suggests that the virus arises *de novo* in the caterpillars and does not arrive from outside sources. He seems, however, to have failed to consider one other avenue of infection and that is the possibility that the virus was transmitted through the eggs. In this connection the following experiment performed by Glaser is of interest. Eggs were obtained from tent moths that had survived experimental larval infections with tent-wilt virus, another polyhedral disease. Before death, or shortly after, all the moths were microscopically ex-

amined for polyhedra or other evidences of the wilt disease, without result. The next spring the egg clusters were "broken up" in order to separate the eggs which are held together with a mucilaginous substance. Twenty-five eggs were then submerged for 10 minutes in a mixture of equal parts of 1 : 1000 corrosive sublimate and 95 % alcohol. This was done in order to destroy any virus which might be adhering to the outside (chorion) of the egg. Experience has shown that the wilt virus is rapidly destroyed by this treatment. The eggs were then washed in sterile water and placed on sterile filter paper in sterile glass phials, one egg to a phial, each phial being plugged with cotton-wool. On hatching, each larva received cherry leaves, selected from a region entirely free from tent larvae, and thoroughly washed before being tendered. In spite of all these precautions three out of the twenty-five larvae so handled developed wilt disease and died in the fourth and fifth stages. Naturally, if all the caterpillars had been confined together, the mortality would have been higher and some would undoubtedly have become infected and survived. Thus it is easily seen how a progression might be obtained with each succeeding generation. From this experiment, Glaser concludes that the only statement one is justified in making is that it seems likely that the virus is transmitted from generation to generation through the eggs of a certain proportion of individuals.

In plants.

Out of the very large numbers of plant virus diseases which have been described we have space only to mention one or two of the more important or outstanding examples. Not long ago complaint was received from a prominent firm of nurserymen that a certain favourite variety of blood-red wallflower had developed unsightly yellow streaks in the petals. It was observed that when seed was saved from plants which bore these yellow-striped flowers and grown in an insect-proof glasshouse, wallflowers were produced which bore normal blood-red flowers. Not so many years ago, this phenomenon would have been explained on the grounds of "unsuitable conditions" or "degeneration". Now, however, it is known that the change or "break" in the flower colour is due to a virus brought to the plant by aphides either from similarly affected wallflowers and stocks or from infected cabbages and other Brassicae. A similar type of virus is common among tulips and causes self-coloured varieties to produce variegated or "broken" flowers. In America certain of these variegated tulips fetched large sums of money before it became known that they were merely virus-diseased specimens of a self-coloured variety. Unfortunately, a tulip once infected with this "breaking" virus is permanently diseased, since in plants which are vegetatively propagated the virus is propagated also. Plants like the wallflower, on the other hand, which are grown from seed come up virus-free because so few viruses are seed-borne.

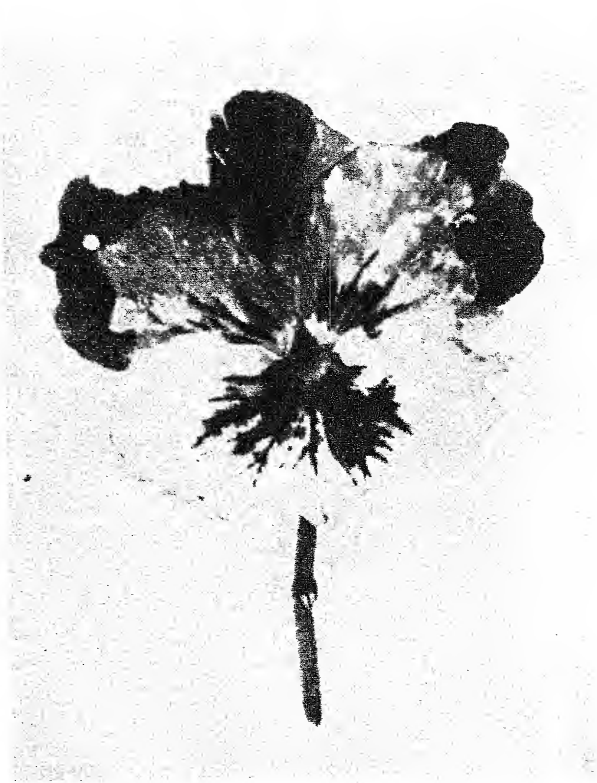


Fig. 15. Flower of viola showing the darkening of the petals caused by infection with cucumber mosaic virus.

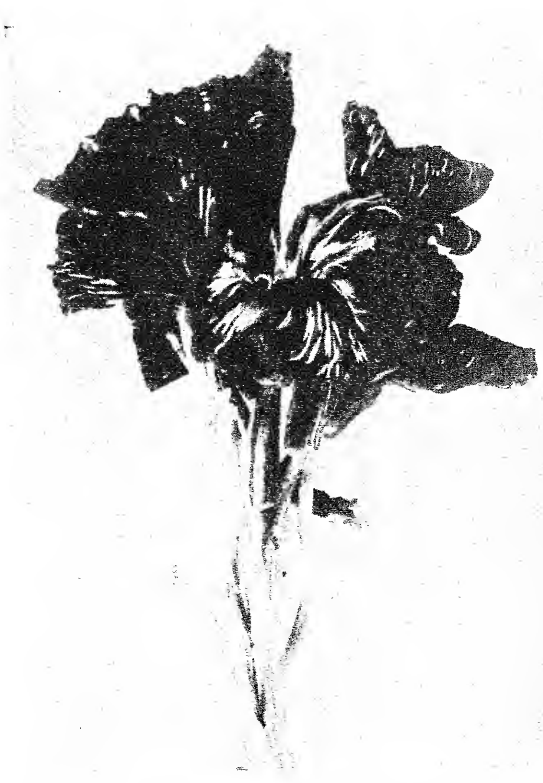


Fig. 16. Red wallflower showing the yellow streaks in the petals induced by a virus brought by green-fly from cabbages.

The classical virus disease of tobacco described by Ivanovski belongs to the "mosaic" group of diseases, in which the symptoms on the leaves take the form of a mottling of yellow or pale green which bears a fancied resemblance to a mosaic pattern. Some of these mosaic viruses produce numerous circles on the leaves of certain host plants; these circles are sometimes single rings but more usually are concentric and generally have a spot in the centre.

Distortions, malformations, and outgrowths of various kinds are characteristic of several plant-virus diseases. A recently described virus disease of the tomato plant exhibits this type of distortion to a high degree. The leaves may be much elongated with only the breadth of a narrow ribbon, or they may be entirely without a lamina and consist merely of long strings of tissue. Other leaves develop long tendrils and appendages, giving the appearance of vegetable spiders. Outgrowths or "enations" in the form of miniature leaves sprout out from the under-surfaces of other leaves, and the whole plant presents a grotesque appearance. Many of these distortions closely resemble genetical abnormalities.

In a virus disease of cranberries in the United States the outstanding symptom is the production of a "false blossom" from which the disease gets its popular name. The calyx lobes of diseased flowers become enlarged, the petals are short and streaked with red and green, and the stamens and pistils are abnormal. Sometimes in severe cases the entire flower may be replaced by successive whorls of leaves or by a short branch.

Asters in the U.S.A. are affected by a serious virus disease called "aster yellows". In this type of disorder there is no "mosaic" mottling, but the plant, or a section of the plant, becomes yellow or chlorotic, as it is usually called. Curiously enough, while the chlorophyll is suppressed in parts of the plant which are normally green, the flowers themselves frequently contain a green-coloured substance. Numerous secondary shoots arise in the axils of leaves giving the plant a bushy appearance, and the leaves stand upright instead of lying flat as in a normal plant. The aster yellows virus cannot be transmitted by mechanical inoculation but is dependent for its spread upon a certain species of leaf-hopper.

In some virus diseases of plants numerous secondary shoots arise producing a dense mass with a bunched appearance. Such growths are called "witches' brooms", and they occur in both herbaceous and woody plants. The witch's broom disease of lucerne is widespread in New South Wales, while a similar type of disease has been observed in the United States affecting an ornamental tree known as black locust. It is not known how these two viruses are spread under natural conditions.

In bacteria.

Before concluding our short account of the virus diseases affecting various kinds of living things we must give a brief description of that apparent virus disease which attacks the smallest of all living things, the bacteria, and which is known as the "bacteriophage" or just 'phage.

In 1915, F. W. Twort observed some curious clear patches developing on a culture of bacteria which were growing on an agar plate. Closer observation of the patches showed that the bacteria in these areas appeared to be destroyed and dissolved. Twort took some of this culture, filtered it through a bacteria-proof filter to remove all living cells and added a few drops of the filtrate to a fresh growth of the same bacteria. In a short time similar clear patches developed in the film of bacterial growth on the agar plate. This experiment shows that the agent causing the destruction of the bacterium is of the filter-passing type.

Two years later the phenomenon was rediscovered by d'Herelle who found, in a filtrate from the faeces of a patient recovering from Shiga dysentery, something which dissolved, or *lysed*, the Shiga bacilli. d'Herelle put a drop of this substance into a fresh culture tube of the bacilli and found that this also soon became clear, showing that the organism had been destroyed. In this manner, starting with a drop or two of the original filtrate, more than a thousand transfers or "passages" were carried out, each transfer involving a dilution of several hundred times. In this repetition of the process, instead of becoming weaker, the activity became more and more pronounced; that is, the disappearance of the bacilli was effected with greater and greater rapidity. At the end the final filtrate was so active that a minute drop of the fluid would completely lyse a culture of several thousand million bacilli. This experiment shows that the agent, or bacteriophage, possesses the power of

multiplication or of increase within living cells, i.e. the bacteria, in a manner similar to viruses.

Twort's discovery made in 1915 was obscured by the clouds of war, while d'Herelle's observations, 2 years later, though also made in wartime, evoked greater interest. This may have been because of the possibility, visualized at the time, of employing this unseen enemy of bacteria to conquer some of the infectious diseases of man. This was the theme of Sinclair Lewis's book *Martin Arrowsmith*, and it will be recalled that the hero set out to defeat a plague epidemic by means of the bacteriophage. Unfortunately, the lysis of bacteria freely exposed in a culture tube or on an agar plate is not quite the same thing as the destruction of harmful bacteria in the human body where they are suspended in a semi-solid mixture of food residues, mucus, etc., and surrounded by enormous numbers of other bacteria. Burnet points out how in cases of infantile dysentery, due to Flexner bacilli, it is possible to isolate, from the same fragment of muco-pus, both a highly active 'phage and normal 'phage-susceptible Flexner bacilli. It is probable that some sort of balance is struck between the two, so that although large numbers of bacteria are destroyed by lysis, equally large numbers survive. At the moment therefore it does not seem hopeful that bacteriophage can be used to combat bacterial disease.

For many years after the Twort-d'Herelle discovery of the bacteriophage a controversy raged as to the nature and origin of this agent. One school of thought, led by d'Herelle, regarded the 'phage as an extrinsic, living and

filterable micro-organism, while another school held to the hypothesis of Bordet who considered it more rational to think the virus did not exist, that the intense action of the bacteriophage represents nothing more than the pathological exaggerations of a normal function connected with mutation and that the lysis is in reality a transmissible autolysis.

Now, during the last few years the bacteriophage has been shown to consist, not of one, but of a whole range of agents, many of which are extremely specific in their action on bacteria. These have been measured and their particle sizes found to range from 10 to 75 mμ or more. Some 'phages have been obtained in pure form and shown to be of a protein nature. In fact, the whole behaviour of the bacteriophage falls into line with that of other viruses, and the controversy about its origin and nature can therefore appropriately be merged in the larger questions of the origin and nature of the filterable viruses as a whole.

Viruses and malignant tumours.

Much consideration is being given by the pathologists of the present day to the possibility that cancer is caused by a filterable virus. It cannot yet be said that any virus has been found associated with mammalian cancer, but certain malignant growths in fowls, mice, rabbits and frogs have been definitely proved to be due to viruses. Since there are tumours in which a virus is known to be constantly present, it seems permissible to suggest that viruses might be associated with all

tumours. As Purdy points out, in the tumours in which no virus can be found the behaviour of the cancer cells within the body of the host is exactly like the behaviour of cancer cells of tumours in which a virus is known to be present.

Certain substances, tar for example, when continually painted into the skin of animals produces a malignant tumour or carcinoma. Now what is the cause underlying the production of the tar carcinoma? Is it due to the action of the tar alone which presumably acts upon the cells causing them to proliferate? or is there some kind of working partnership between the tar and a virus stimulated into action by the tar? It does not appear possible to transmit a tar carcinoma from host to host by means of a cell-free filtrate, but this need not necessarily imply that a virus is not present. The Rous sarcoma, which is known to be caused by a transmissible filterable virus, appears sometimes to have a non-filterable phase. In other words, filterability may be lost for a time and then regained. In some interesting work on a non-filterable tar sarcoma, Andrewes showed that if pheasants were inoculated with an extract of this tar sarcoma, they all developed antibodies which would neutralize the virus of the Rous sarcoma. This seems to be fairly good evidence that the non-filterable tar sarcoma contained a virus which was responsible for calling forth the antibody production in the inoculated pheasants, and this virus must be closely related serologically to that of the Rous No. 1 sarcoma.

Some recent work of the Mellanbys on the Rous

sarcoma does not seem to support this theory. They were unable to propagate a chemically-induced tumour by cell-free filtrates. If, however, a fowl with a chemically-induced tumour is injected with Rous virus, then cell-free filtrates of this tumour will give rise to Rous sarcomata in other fowls. On the other hand, if cells of this same tumour are grafted into fowls, then tumours resembling the original chemically-induced tumour are produced. These facts suggest that chemically-induced tumours do not contain a virus similar to that causing the Rous sarcoma. It is an interesting fact that when the Rous virus is injected into the blood of a fowl, the virus seems to become distributed throughout the blood and organs in the body but does not produce tumours. If, however, a muscle is injured immediately after the intravenous injection of the virus, then a tumour develops at the point of injury, but if the injury is made before injection of the virus, then no tumours are developed.

There seems to be some close connection between injury to the cell and the development of tumours, and this is well illustrated by the reaction of tarred tissues with viruses. There is indeed considerable evidence that viruses and chemical carcinogens, such as tar, act in concert in causing new growths. It may be that these agents act on the cell in such a way as to facilitate the entrance of the virus into the cell. We can illustrate this by reference to the important work of Rous and Kidd on the Shope rabbit-papilloma virus. This virus produces a wart-like condition in rabbits which in time will often

become cancerous. In these experiments, one ear of a series of normal rabbits was tarred at intervals until small warts began to appear in the tarred area. Then virus from a Shope papilloma was injected into the normal untreated ear of the tarred rabbit. A rapidly growing tumour promptly appeared in the tarred area, and this tumour appeared much earlier and was more active than tumours which are produced either by tar or virus acting alone. It is evident that the virus tends to localize itself in the tarred area where it causes the cells to proliferate. When the virus failed to localize in the ears of injected rabbits no malignant growths developed.

There appear to be two factors necessary for the production of these malignant growths, one is the localization of the virus in the tarred area and the other is some preparatory change of the epidermal cells which become infected with it. Rous and Kidd have also found that tar tends to elicit a different type of tumour according to the kind of animal treated. For example, tarring the skin of dogs evokes a type of malignant growth seldom if ever evoked by tar in the rabbit or mouse. Concerning this phenomenon, Rous and Kidd ask: "Can it be that the epidermis of different species of animals has different inherent potentialities for tumour formation? Or are these potentialities not inherent but due to agents of extraneous origin? Such questions can only slowly be answered."

Somewhat similar results have been obtained by Ahlström and Andrewes using the fibroma virus on

tarred rabbits. At first the reactions of the tarred and normal rabbits to the virus are identical, but after 2 or 3 weeks a difference is apparent. The lesions in the normal rabbits cease to grow and then regress, but those in the tarred rabbits continue to grow for some time longer and their regression is delayed for several weeks. The suggestion is made that the tar damages the mechanism which normally calls a halt to the proliferation and soon leads to regression of the tumours.

It is clear that research into the problem of viruses in relation to the growth of malignant tumours is only beginning, though the results already achieved are significant enough. In a recent presidential address to the British Association Sir Henry Dale said: "It is impossible, to anyone having even a slight knowledge of the recent developments which began with the work of Rous and Murphy, to doubt that in the advance of knowledge concerning the nature of viruses in general lies the brightest hope of finding a clue to the dark secret of the malignant tumours."

CHAPTER VIII

PREVENTION AND CONTROL OF VIRUS DISEASES

We have seen how the pioneer work of Jenner with smallpox virus and that of Pasteur with rabies virus showed the way to immunity from these diseases. Before we consider further the methods of prevention and control of virus diseases it will be as well to explain shortly the various types of immunity that are known.

When a person or animal has suffered from an attack of infectious disease it frequently happens that a *natural immunity* develops which prevents a recurrence of that disease for some time. In virus affections the subsequent natural immunity is very strong, and in the case of certain viruses affecting man, one attack of the disease confers a lifelong immunity; chicken-pox and smallpox are two examples of this. Indeed the immunity conferred by many virus infections against a second attack of the same agent is so strong that it is considered by some authorities to be in a different category altogether from the immunity which is subsequent to bacterial diseases. There are, however, some viruses which are exceptions to this rule, the immunity conferred by attacks in man of herpes simplex, the common cold and influenza is very transient, and the same is true to a less degree of foot-and-mouth disease in cattle. The problem is complicated in the last two cases by the existence of

numerous strains of the viruses which do not immunize against each other.

The immunity which develops in an animal to a second attack of a specific bacterium or virus is due to the formation in the blood or body fluids of a substance or property known as an "*antibody*". This antibody has the power of neutralizing the specific bacterium or virus which is known as the *antigen*. Now the principle underlying the work of Jenner and Pasteur was to stimulate the formation of antibodies without the necessity for the organism to undergo the painful and dangerous ordeal of having first to suffer the disease. It will be remembered that the cow-pox or vaccinia used by Jenner in his inoculations is a mild strain of the smallpox virus which, although only producing a slight local disease, yet is sufficient to immunize the body against the much more virulent virus of smallpox. Similarly, Pasteur used an attenuated or weakened strain of rabies virus which he obtained by drying the spinal cords of infected rabbits.

The reader will now understand the importance of any process, such as the tissue culture methods described in Chapter VI, which changes a virus so that its virulence is reduced.

Another method of inducing immunity is by inoculation with viruses which have been inactivated or "killed" by various methods but which still retain their antigenic properties, or in other words are still able to stimulate production of antibodies. Such "killed" viruses are usually spoken of as *vaccines*.

In some cases immunity is conferred by inoculating

a weak strain of a given virus together with antiserum (antibody) from an animal which has recovered from the disease. This reduces the risk of the weakened virus giving rise to serious disturbances in the body.

The foregoing conditions where the disease agent itself in one state or another is used to induce immunity are spoken of as *active immunity*. There is, however, another method of inducing immunity in which serum from a convalescent patient is used as the protective agent. This serum contains the antibodies or immune bodies produced against the particular disease in question. This type of protection is known as *passive immunity* and has been largely used, for example, in preventing measles epidemics. This method of immunization has its disadvantages, since it is not always easy to obtain serum in sufficient quantity, and also rigorous tests are necessary to ensure that other infectious agents are not also present in the serum used. For these reasons much work has been done in attempting to find a suitable animal which could be utilized for the preparation of antisera.

In studying the virus of epidemic influenza it was necessary to find some small animal, cheaply and easily reared, which could be used in the experimental work. After testing various animals of this type it was discovered that hedgehogs, mice and ferrets were all susceptible to the influenza virus, and much of our knowledge concerning the immunological responses to infection or vaccination with this virus has been obtained by experiments on the two latter animals. Here

it may be remarked that one of the workers actually contracted influenza from an inoculated ferret which sneezed in his face. This shows that the ferret virus has retained its infectivity for man, while it is possible that the mouse strain may have lost it, since no case of infection from mice is known in spite of close contact between workers and infected mice.

One of the many difficulties which confront the investigator is the existence of febrile conditions which much resemble influenza but which do not yield a virus pathogenic to ferrets. Some recent work suggests that in the future it may be possible to isolate strains of influenza virus from man by employing mice or chick embryos in the place of ferrets, and this would remove one of the great difficulties of laboratory diagnosis.

The Medical Research Council has recently issued a special report on the study of epidemic influenza, and in describing what is being done toward the control of the disease we quote freely from this report.

Attempts have been made to treat epidemic influenza by means of antiviral horse serum. The serum was prepared by inoculating a horse with material from a ferret infected with human influenza virus and concentrating the resulting serum eight times. This serum had the power of inactivating or destroying the virus when mixed with it, and also increased the survival rate of mice which had been previously infected with the virus. Inoculations were made to a number of human volunteers suffering from epidemic influenza, but the results were inconclusive. To obtain a conclusive result it would

be necessary to have a large series of treated cases with strictly alternate control cases and these were not forthcoming.

One of the great difficulties to be faced in dealing with prophylactic measures against influenza is the existence of numerous strains antigenically quite distinct. This means that a vaccine or antiserum prepared against one strain offers no protection against another strain of the same virus.

Vaccination of human volunteers with a formalized vaccine of the W.S. virus strain has been carried out on a fairly large scale. Here again, however, the results were inconclusive largely because the vaccinations were done after the epidemic had commenced, and an interval of 14 days between vaccination and exposure is required before a just conclusion as to the value of such vaccination can be drawn. There were, however, four cases of virus influenza in volunteers who had been vaccinated more than 14 days previously, and in at least one case the virus strain recovered from a vaccinated subject was not the same as that used in the preparation of the vaccine.

The large amount of careful work that has been carried out on the control of influenza has given promising results, and it is possible that the difficulties inherent in dealing with the many strains of influenza virus may be overcome. There are indications that these strains overlap to a considerable extent, and a strain may be found which will cover all the types sufficiently well to render unnecessary successive inoculations with vac-

cines made from three or four strains. Moreover, there is also the possibility that cultivated influenza virus may be used for vaccination purposes, and Burnet has shown that after thirty or forty consecutive passages a strain of virus may be developed which is highly pathogenic for the developing egg. The chorion-allantoic membrane of the chick embryo may therefore prove a valuable medium for this purpose. In America Shope has succeeded in immunizing swine against swine influenza by injecting active virus intramuscularly or subcutaneously. The virus was obtained from the lungs of infected ferrets or mice, and when injected in this way seems to confer immunity without causing any disease manifestations.

We have seen in a previous chapter how the virus of yellow fever can be grown in cultures consisting of chick embryos from which the nervous tissue has been removed. Virus cultivated in this manner loses its affinities for nervous tissue and becomes greatly reduced in virulence. It is now possible to use this weakened cultivated virus to immunize persons who are likely to be exposed to the risk of yellow-fever infection.

In studying yellow-fever epidemics in rural areas, one puzzling feature has been the manner in which the virus "tides over" the dry season when the mosquito vector is absent or very scarce. A similar puzzle confronted the investigator of a very important virus disease of cotton in the Sudan called "leaf curl" which is transmitted by a small plant-sucking insect known as a "white-fly". Now during the dry season in the Sudan the white-fly entirely disappears, yet every time the new cotton crop

was raised, the new generations of white-fly were able to infect it with the virus. The puzzle was solved by the discovery that the virus persisted in the "ratoon" cotton, the plants which survive the dead season and produce new growth, nearly always diseased, when the land is irrigated for the sowing of other crops. In the case of yellow fever, however, one can hardly conceive of a reservoir of this virus in a plant, and some other explanations must be sought. Now it has been shown that yellow-fever virus will persist for quite long periods inside the bodies of insects which play no part in the transmission of the virus, cockroaches for example. Further, it is known that monkeys, the normal reservoirs of virus infection, can be infected with yellow fever via the alimentary canal, and monkeys supplement their diet by eating various kinds of insects, including cockroaches. Findlay and MacCallum have recently made the interesting suggestion, therefore, that the virus may survive the "dead" season inside the bodies of insects of a nature quite different from the mosquito. These would presumably be eaten by monkeys which would become infected with the disease.

When we come later in this chapter to consider methods for the control of plant-virus diseases we shall see that it is important to prevent, so far as possible, the arrival of insect vectors bringing a particular virus with them. Now a similar problem exists as regards the mosquito-borne virus of yellow fever. With the development of the aeroplane the danger has arisen of an infected mosquito being carried from a country where

yellow fever exists to some country such as India which is at present free of the disease. In India all conditions for a disastrous epidemic exist—a large human population, susceptible monkeys and the appropriate mosquito vector. To meet this danger, investigations have been carried out to find some chemical which could be used to destroy such unauthorized passengers on aeroplanes as infected mosquitoes. Spraying or fumigation is now carried out as a routine precaution with any aeroplane which arrives from countries where yellow fever exists.

In attempting any kind of vaccination process of animals against foot-and-mouth disease investigators are faced with the same problem as in the case of influenza, a multiplicity of strains which do not immunize against each other. In the virus of foot-and-mouth disease, however, the strains seem more sharply distinct than in the influenza virus. In the British Isles the policy has always been to control the spread of the disease by immediate slaughter of infected stock and possible contacts and through disinfection of the premises. Nevertheless, search for a satisfactory method of immunization is being actively carried on in many countries at the present time.

Recently a vaccine against foot-and-mouth disease has been produced in Copenhagen, and this is being tried out in Germany on a small island off the south coast of Rügen. About 50,000 cattle have been inoculated and the immunity conferred seems to last not less than 3 months. It is hoped that the vaccine will protect against the three main strains of the foot-and-mouth

virus. At present the production of the vaccine is rather expensive, since one cow provides only enough material to inoculate twenty-five cattle. There is a hope, however, of reducing the cost by cultivating the virus, since it can be grown in the skin of the embryos of the cow and the sheep. It has been observed that during the recent epizootic of foot-and-mouth disease in Germany that the inoculated cattle escaped infection while pigs on the same farms contracted the disease. At the same time it should be remembered that outbreaks sometimes occur which are confined to pigs. This may be due to the occurrence of a variant of one of the known virus strains.

We come now to consider what has recently been done to combat another scourge of animals, the virus disease of dogs known as dog distemper. In Britain the incidence of the disease in young foxhounds, for example, is or was, nearly 100 %, and the mortality rate is frequently 50 % and may on occasions exceed 75 %. The very real advance which has been made in the control of this extremely deadly disease is due to the careful work of Dunkin and Laidlaw, and in this account we quote freely from their publications. Just as in the work against influenza and foot-and-mouth disease, it was necessary to have some small experimental animal for making tests with the distemper virus. For this the ferret again proved suitable and was used extensively in the work. It was early noticed that ferrets which had once recovered from an attack of dog distemper developed a solid immunity from a further attack. The next step was

to prepare a vaccine, and for this tissue with a high virus content was necessary.

It was discovered that the spleens of infected ferrets fulfilled this condition, and the next step was to inactivate or "kill" the virus without affecting its antigenic properties, in other words, without destroying its power to confer immunity. A number of experiments were carried out with virus inactivated by heat, phenol and formaldehyde, and the best results were obtained with formaldehyde. The vaccine finally used therefore consisted of the formalized spleen tissue from infected ferrets, and 2·0 c.c. of this as a standard dose protects a ferret completely from a dose of active virus. An interval of 10–14 days between injection of vaccine and exposure to infection is necessary for protection, and the immunity thus given is of long duration, but to consolidate it a dose of active virus must also be subsequently inoculated. When this ferret-distemper vaccine was tested on dogs, however, it was discovered that in order to be effective multiple injections had to be given.

Dunkin and Laidlaw next turned their attention to the production of a vaccine from distemper-dog tissue, and again it was found that the spleen was unusually rich in virus as was also the liver. A formalized vaccine was therefore prepared from these organs which gave immunity to dogs, and this immunity had to be consolidated by subsequent injections of active virus. Incidentally this distemper vaccine prepared from infected dog tissue conferred little or no protection on the ferret against the virus.

So far we have been discussing the active immunity conferred by the use of vaccines; we come now to the possibility of inducing passive immunity to distemper by the injection of antiserum. The distemper antiserum was prepared in the following manner: Dogs which had recovered from an attack of distemper or, better still, those which have been artificially immunized are kept for at least a month until all symptoms have subsided. Next a supply of virus is secured from an active case of distemper in a dog, using the spleen and lymph glands as the source of virus. 20 c.c. of a 20 % suspension of this virus are then inoculated with strict aseptic precautions into the dog to be hyperimmunized. On the following day another dose of virus of the same size is injected on the side of the body opposite to that receiving the first injection.

The reason for this double injection of a large quantity of active virus is the desire to stimulate the mechanism of the "antibody factory" with as powerful a stimulus as possible.

About the fifth or seventh day after the second injection the animal is bled, the blood is allowed to clot and the immune serum is collected.

To immunize a dog against distemper by this method a simultaneous injection is given of active virus on one side of the animal and of immune serum on the other. Mixtures of serum and virus injected together do not confer immunity.

This method of immunizing dogs is recommended as being more advantageous than the vaccine-virus method.

First of all it necessitates only one visit from the veterinary surgeon which is a point of some practical importance, and secondly, there is perhaps less risk to the dog because if there is a rise of temperature in the animal on the fourth or fifth day following inoculation, a dose of serum may be injected at once and the threatened trouble suppressed. On the other hand, it is possible that the immunity conferred by the antiserum-virus treatment may not be quite so solid as that following inoculation with vaccine and virus.

While dog-tissue vaccines offer little protection to ferrets against distemper, immune serum from dogs does give some immunity. If, however, the disease has already started in the ferret, doses of dog distemper antiserum, even if massive, have no beneficial effect on the disease. On the other hand, in the case of dogs, considerable benefit is derived from doses of serum even after the initial rise of temperature in an actual attack of the disease.

Efforts have been made to follow up the after-history of inoculated hounds, always rather a difficult matter. This has been done, however, with hounds belonging to twenty-three packs and inoculated during the years 1928 and 1929. Of 750 hounds originally inoculated, a certain number had been drafted to other packs and their after-history lost; there still remained, however, 650 hounds which are known to have been exposed to distemper infection at least once and sometimes several times. Of these more than 80 % were still alive and immune to distemper some 2 years afterwards.

This is clearly an excellent result, and we can hardly doubt that a great step forward in the control of the disease of dog distemper has been made. So far as is known at present the distemper virus does not occur in numerous different strains as is the case with the influenza and foot-and-mouth viruses, so that one difficulty in the preparation of vaccines and antisera is avoided.

One of the important events in medical science has been the recent discovery of the germicidal action of a dye known as prontosil, one of the sulphonamide group of drugs. This substance not only destroys bacteria in the test-tube but also in the human body and so fulfils the hope once centred in the bacteriophage. Prontosil is particularly useful because of its action against such organisms as streptococci and pneumococci, which cause deafness, pneumonia and other diseases in man. The effect of the prontosil is apparently to dissolve the outer capsule of these bacteria which are then effectively dealt with by the blood.

The next step will be to try this type of chemotherapy on the virus diseases, and not much work has yet been done from this aspect although a start has been made. Prontosil has been tried against dog distemper, and although some benefit has been claimed it is possible that this may only be due to the destruction of bacteria in a secondary infection. MacCallum and Findlay have experimented with sulphanilamide and one of its allies on a virus disease of mice, and they conclude that these compounds have the power of protecting a con-

siderable percentage of mice against active strains of the virus.

From even this brief survey of some of the results achieved in the incessant war against the viruses it is clear that we have worthy successors to Jenner and Pasteur and that we can hope for achievements equal to theirs. The pity is that so much treasure should be poured out by all the nations of the world to produce armaments with which to fight each other while the real enemies of mankind are still unconquered.

In the preceding short account of the efforts to control virus diseases of man and animals it will be seen that emphasis is given to immunizing the individual against infection. Since there is no acquired immunity in plants comparable to that obtaining in animals, the methods of controlling plant-virus diseases have of necessity developed along different lines. The problem can be approached from two standpoints, the first aim being to start with a virus-free crop and the second to keep it virus-free during the growing period. In the discussion on the methods of spread of plant viruses it has been pointed out that viruses are seldom carried in the true seed. There are exceptions to this rule, the common mosaic disease of beans being a case in point, but, on the whole, seed transmission of plant viruses is a rare occurrence. On the other hand, where a plant is multiplied by some form of vegetative propagation, by tubers, sets or cuttings, then it is a matter of first importance that the parent plant should be free of virus, otherwise the disease will be propagated anew. It is this fact which

gives an added significance to the virus diseases of such a crop as the potato, which is propagated entirely by vegetative means.

Every English farmer knows that if he continues to save his own "seed" potatoes for several years from his own crop, the yield per acre will decrease rapidly in each succeeding year. Not long ago this was spoken of as "degeneration", and it was thought to be due to a kind of senile decay following upon prolonged growth by vegetative means. Now it is known that this degeneration merely represents progressive contamination with virus disease and has no connection whatever with senile decay induced by continued vegetative propagation. To avoid this state of affairs, then, the English farmer buys his "seed" potatoes afresh each year from Scotland, because he knows that by doing so he will at least *start* with a crop of potatoes comparatively free from virus infection.

In the case of many other crops, vegetatively propagated, it has become the practice in certain countries to maintain, either in isolation or by careful surveillance, virus-free stocks from which the propagating material is derived. This system has been applied among others to potatoes, raspberries and bananas. In America an elaborate "tuber-indexing" system has been developed whereby a percentage of a stock of "seed" potatoes is forced into growth during the winter and tested for their virus content. By this means an estimate can be formed of the amount of virus present in a stock of tubers prior to planting.

Assuming then that by one or other of these methods the crop is enabled to commence growth in a virus-free condition, the second part of the problem is to prevent the entry and subsequent spread of virus. This part of the control problem may be approached from several aspects. Since so many plant viruses depend upon insects for their dissemination, any measures directed against the insect vector will help to prevent the spread of the disease. The production of resistant varieties of plants is a promising line of work and it is one which is already giving fruitful results. Finally, there is a method of "vaccinating" plants against infection, although strictly speaking this is not an exact parallel to the vaccination we have described in dealing with animal virus diseases.

It is seldom possible to destroy the insect vectors of viruses affecting agricultural crops by direct means such as the application of insecticides, though under glass-house conditions insect vectors, like aphides and thrips, can be kept down by regular fumigation. On the field scale, however, the insect is better dealt with by cultural methods such as the destruction of those weeds which may act as alternate host plants or by the selection of localities which are unsuitable for the movement of the insects. The application of this latter method to the growing of virus-free "seed" potatoes was developed by the work of the late Maldwyn Davies. He found that high humidity greatly reduces the flight of the aphis, which transmits the virus diseases of the potato, and in consequence prevents their spread. He also found that

the aphides are unwilling to take to flight when the velocity of the wind exceeds 3–4 miles per hour. These findings suggest therefore that the best localities for growing "seed" potatoes are not necessarily at high altitudes with bleak exposed conditions, as hitherto supposed, but may be quite low-lying and often at sea-level.

The serious virus disease of sugar beet known as "curly-top" is entirely dependent upon one species of sap-sucking insect, a leaf-hopper, for its dispersal, and, in consequence, the numbers and movements of this insect are of supreme interest to the growers of sugar beet. The reasons underlying the annual migration of this insect into the sugar-beet fields are not entirely clear. If, however, conditions were favourable for hibernation the previous autumn, an extremely dry spring will prove disastrous, because under these dry conditions the alternate host plants of the leaf-hopper are killed off or do not germinate. This results in wholesale migration of the leaf-hoppers to the beet fields. Another contributory factor to this movement of the leaf-hopper in America is the over-grazing of the foothills, leading to destruction of the natural vegetation and consequent soil erosion. The natural vegetation has been replaced by an invasion of the Russian thistle *Salsola kali*, which harbours large numbers of the leaf-hopper. The loss therefore from curly-top disease of the sugar beet depends upon the numbers of leaf-hoppers which invade the beet fields. In normal seasons this loss may be comparatively small, but when the insect numbers are large the virus spreads

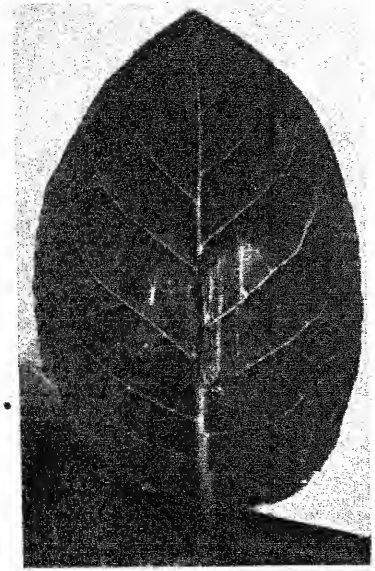


Fig. 17. This photograph illustrates the multiplication of the virus near the site of inoculation on a tobacco leaf: note the lesions forming near the rubbed area.

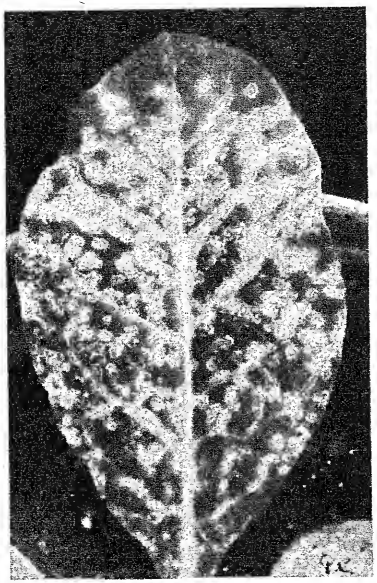


Fig. 18. A virus from potatoes which produces rings on the tobacco leaf.

Fig. 19. Example of unequal growth caused by virus infection: the left half of the leaf has ceased growing and this has set up stresses which cause the leaf to bend over and then to tear.

at a rapid rate and the yield may fall to one-tenth of the normal.

In some cases considerable success has attended the efforts of plant breeders to produce virus-resistant varieties of plants. Perhaps the most outstanding success was the breeding of the P.O.J. varieties of sugar cane which are resistant to the mosaic disease of this plant. The introduction of these resistant varieties has rehabilitated the industry in countries where the disease had threatened its extinction. In America several strains of sugar beet have been evolved which show a fairly high degree of resistance to the destructive curly-top disease. Some success has also been attained in the Sudan with a variety of cotton resistant to a virus disease known as "leaf-curl".

When discussing in Chapter II the formation of "local lesions" by viruses on the leaves of plants, we have seen that a species of *Nicotiana*, *N. glutinosa*, reacts to infection with tobacco-mosaic virus with the formation of those local lesions on the leaves without any further spread of the virus. Such a plant is quite healthy except for the slight local infection in the leaf. Now it has been shown by some interesting work by Holmes in America that this power of localizing virus in the leaves is an inherited factor. It is hoped therefore to produce a variety of tobacco containing the gene which confers this power of localizing the virus. If such a tobacco plant could be evolved which was at the same time commercially profitable the advantages would be very considerable. Tobacco mosaic is an almost universal disease,

and it has a very deleterious effect on the tobacco leaf from the grower's point of view. The virus of this disease is spread entirely by mechanical contact between healthy and affected plants and by the ordinary processes of tending the plants. If therefore the virus was localized in a few small areas of the leaf, the injury to the plant and the chances of transmission would both be reduced to a minimum.

We have mentioned the possibility of controlling certain plant-virus diseases by a process analogous to "vaccination". It is necessary that the reader should clearly understand the difference between this kind of inoculation and the vaccination of animals described at the beginning of this chapter. In the latter case the body responds to vaccination by the production of antibodies which confer immunity to the disease in question. In plants, however, there is no evidence at present of the production of antibodies, and the only acquired immunity that is known depends on the presence of a virus already there. Before we can "vaccinate" a plant, therefore, against a particular virus there must exist a "weak" and a "strong" strain of this virus. It is the presence of the weak avirulent strain systemically spread throughout the plant which protects it from invasion by the more virulent strain. In other words, we give the plant a mild disease in the hope of protecting it against a severe one; it seems to be a case of "first come first served". This protection only holds good for like viruses and virus strains, a weak strain of one virus affords no protection whatever against the entrance of either a

weak or strong strain of a different virus. At the moment the interest of this method of plant protection is largely academic, but it does seem to offer scope for further development.

In certain bacterial diseases of man it is possible for some individuals to bear the pathogenic organisms in the body without themselves showing any signs of disease. Such individuals are known as "carriers" and are of course a danger to the community because they are potential sources of infection. A parallel phenomenon exists in the virus diseases of plants, and the virus carrier is well known to all students of the subject. There are several kinds of virus carriers in plants, and we may divide them loosely into three types: (1) plants which show symptoms at the time of infection and for a few days afterwards but which then grow out of the disease while still retaining the virus in the sap; (2) plants which carry the virus without at any time showing disease symptoms; (3) plants which carry a virus peculiar to that variety and one which does not spread to another variety at all except by artificial methods of transmission. Plants in the first two groups may serve as sources of infection to other neighbouring plants, but the virus carried by plants in the third group appears not to spread at all by natural means. Virus carriers occur frequently among solanaceous plants and particularly among different potato varieties. The common potato mosaic, or *X*, virus is present without symptoms in many different varieties of potato. Indeed, so frequently is it carried without symptoms by American

potato varieties that it is known in that country as the "healthy potato virus" or "latent virus".

Symptomless virus carriers also occur in certain varieties of the hop plant, such plants carrying the virus of hop mosaic and possibly other hop viruses. It is therefore unwise to grow these carrier varieties of hops close to other intolerant varieties.

In our third group of carriers we put those plants which carry a virus which seems peculiar to the carrier plant itself. This kind of carrier is only known in the potato, but it is possible that examples may occur in other crop plants. The potato variety King Edward is universally affected with a virus which does not in any way affect the health of the plant. This is shown by its hardiness and vigour and the way in which it holds its own with other newer potato varieties. Nevertheless, if a scion from a normal King Edward potato plant be grafted on to certain other potato varieties, such as Arran Chief, it induces a severe virus disease in the stock plant. It is very interesting to find that all plants of King Edward from any part of the world are invariably infected with this virus. However, the virus does not spread in the field to other potato varieties, there seems to be no insect vector and it can only be transmitted by grafting. Once the virus has been passed on to a susceptible potato variety it can be transmitted indefinitely in series from plant to plant but only by grafting. This shows, however, that multiplication of the agent takes place, just as with any other virus. It is very difficult therefore to imagine the circumstances

under which the first King Edward seedling became infected.

Practically nothing is known of the facts which allow a plant to become a virus carrier or what kind of a balance is struck between the plant and the virus which allows the latter to multiply without causing any disease. It is, however, a very interesting phenomenon, and more fundamental study of the relationship between virus and cell may eventually throw some light on the carrier problem.

It will not perhaps be out of place in this chapter to comment briefly on the economic significance of virus diseases and to show something of the losses caused to mankind, of life itself, of cattle, and of crops by these insidious agents of disease.

Before the advent of vaccination the number of deaths from smallpox was appalling, in Russia alone 2,000,000 people are said to have died of the disease in one year. We may therefore appropriately make the following quotation from *Nature* of 22 October 1938: "Smallpox has become an extinct disease in Poland owing to the strict enforcement of the law on compulsory vaccination and re-vaccination."

The great influenza pandemic of 1918–19 caused the loss of more lives than did the whole of the Great War. In London alone there were over 18,000 deaths from the disease. Every year there is a varying amount of illness and disability directly attributable to this recurring annual plague.

The losses caused by foot-and-mouth disease to

farmers and to Governments, not only in this country but practically all over the world, are prodigious and amount to several million pounds a year. Laidlaw points out that in the last big epizootic of this disease, by the end of 1937, there had been at least 130,000 outbreaks in France, 63,000 in Belgium, 100,000 in Holland and 36,000 in Germany. Each of these outbreaks involved considerable numbers of animals, so that the total number of cattle concerned during the year must have been extremely large.

In England and Wales about half a million acres are planted annually with potatoes. Of this area some 380,000 acres are planted with local "seed", much of which is infected with virus disease. It has been calculated that the use of fresh "seed" raises the yield by 1 ton per acre, so that on the basis of a valuation of £5 a ton the loss on 380,000 acres of potatoes due to virus disease alone is not far short of £2,000,000.

Curly-top is a serious virus disease of sugar beet in the United States, and the use of varieties of sugar beet in California which are partially resistant to the virus has led to a gain of between 2 and 3 tons per acre. This is calculated to give a saving on 102,000 acres of 1,657,000 dollars.

As regards the mosaic disease of sugar cane, Coons has shown that in Louisiana alone the introduction of disease-resistant varieties of sugar cane raised the yield by approximately 50 %. The annual benefit from the use of these resistant varieties is calculated to be nearly 6,000,000 dollars. The situation as regards the virus diseases of strawberries and raspberries is now very

serious. Since both these crops are vegetatively propagated, they are extremely liable to become progressively infected with viruses, and several excellent varieties of raspberries have fallen out of cultivation for this reason alone. It is not uncommon to read in the catalogues of prominent fruit growers some such notice as this: "We regret we can no longer recommend this variety of raspberry, owing to its infection with virus diseases."

Not long ago the banana industry in Australia was threatened by a virus disease known as "bunchy top", and disaster was only averted by the ruthless eradication and destruction of infected plants and by other methods of control.

In England the hop growers are seriously perturbed by a suspected virus disease of hops known as "nettlehead", and at a recent meeting held to consider the situation a sum of money was voted to pay for an investigation of the nettlehead disease.

Just now the news comes from America of an apparent virus disease which is killing many trees of American elm in Ohio. This is not the same as the so-called "Dutch elm disease" which is due to a fungus. In 3 years this suspected virus has killed 1000 out of 1800 elms in Chillicothe, and it is spreading among forest trees in west Virginia, northern Kentucky, southern Indiana and Illinois.

The losses due to the various virus diseases which we have quoted, and which can be taken as reasonably accurate, may serve to give some slight idea of the importance of these disease agents and justify both the title of this book and the quotation on the title-page.

APPENDIX

LIST OF SOME IMPORTANT VIRUS DISEASES

Man
- Yellow Fever
- Warts
- Herpes
- Smallpox
- Chicken-pox
- Acute anterior poliomyelitis (Infantile paralysis)
- Encephalitis lethargica (Sleepy sickness)
- Measles
- Mumps
- Epidemic influenza
- Common cold

Cattle
- Cow-pox
- Foot-and-mouth disease

Horse
- Horse-pox
- Equine encephalomyelitis
- Infectious anaemia

Dog
- Rabies
- Distemper

Rabbits and Guinea pigs
- Infectious myxomatosis of rabbits
- Virus III of rabbits
- Salivary virus of guinea pigs
- Guinea pig paralysis

Mice
- Infectious ectromelia

Birds
- Fowl plague
- Fowl-pox
- Psittacosis
- Filterable tumours

Fish
- Carp-pox
- Lymphocystis disease

Amphibia
- Transmissible tumour of frogs

Insects
- Sacbrood of honey bees
- Polyhedral diseases of caterpillars

Bacteria
- Bacteriophages

Plants
- Curly-top of sugar beet
- Leaf-roll of potato
- Mosaic of tobacco
- Yellows of peach
- Leaf-crinkle of cotton
- Spike disease of sandal

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